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ADJUSTMENT OF IRON SUPPLY TO REQUIREMENTS OF SOY BEAN IN SOLUTION CULTURE[1]

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(WITH TWO FIGURES)

It has long been understood that green plants deprived of iron lack the ability to form chlorophyll, through the agency of which they are enabled to elaborate carbohydrates from carbon dioxide and water under suitable environmental conditions. Although iron is not a constituent of chlorophyll (**24**), GRIS (**8**) more than three-quarters of a century ago showed the necessity of its presence in green plants for the formation of chlorophyll. The significant fact that green plants, grown in culture media without iron, become chlorotic as soon as the reserve iron in the seed is exhausted, is, of course, too well known to need comment.

Quantitative investigation of the frequently observed occurrence of iron chlorosis in plants grown in substrata containing iron has been largely neglected, perhaps on account of its complexity. This somewhat baffling phenomenon, however, has led to considerable speculation as to causes, and, indeed, to some constructive research.

HOFFER and CARR (**10**) found iron accumulation in the nodes of diseased corn stalks, and they state that with the upper leaves

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chlorotic these plants contain considerable amounts of immobile iron compounds. McCALL and HAAG (16), working with wheat, state that some indirect evidence was obtained indicating that plants grown in culture solutions having P_H values ranging from 4.02 to 7.0 sometimes suffer from lack of available iron, or from faulty metabolism resulting from the immobility of the iron within the plants. ARNDT (1), working with corn, found that iron salts when present in injurious concentrations produce a reddish brown discoloration of the lower portions of the nodal areas, owing to an accumulation of an iron combination.

It has been found that great care must be exercised in the use of ferrous salts as sources of iron for plants grown in culture solutions. MAZE (15) found ferrous sulphate, used as a source of iron, toxic to corn. HARTWELL and PEMBER (9) likewise found ferrous sulphate toxic to barley and rye seedlings in Knop's solution, and RUPPRECHT (17) showed ferrous sulphate in a culture solution, when present in concentrations of four parts per million, to be toxic to clover seedlings. JONES and SHIVE (11), employing different increments of iron in the form of ferrous sulphate, found that with some culture solutions it gave excellent results when supplied in relatively very small quantities. These workers showed that in a solution containing ammonium sulphate, the ferrous form of iron is very toxic to plants at exceedingly low concentration, and point out (12) that plants show a marked difference in their response to this form of iron in different types of culture solutions. They further (13) point out that the availability and efficiency of a given iron compound appear to be determined in large measure by the composition of the culture solution, and by the nature of the reaction change induced in the medium by contact with the plant roots. Soluble ferric phosphate has been used with success by DUGGAR (5) for seed plants, and in this connection THATCHER (20) remarks that "only soluble ferric compounds seem to serve as a suitable source of supply of the element, ferrous compounds being usually toxic to plants." Ferric tartrate was found by GILE and CARRERO (6) to be especially available to rice plants, and they have also shown in their work with solution cultures that the amount of iron used has a marked influence upon the availability of this element for rice

plants. Ferric glycero phosphate has been used by MARSH (14) with considerable success in culture media.

TOTTINGHAM and BECK (22) point out that the plant's response to iron during the early stages of growth is dependent upon the amount of iron stored in the seed. CORSON and BAKKE (4) studied both ferrous and ferric phosphates as sources of iron for plants in culture solutions, and found marked differences in their efficiency to supply available iron, and noted also marked differences in the response of different species toward these compounds.

The purpose of the investigations described in the following pages was, in general, to study the relation of the growth of the soy bean to the essential element iron in several types of culture solutions. Studies were made of the conditions under which normal growth of this plant may occur in relation to the iron in the culture medium, and under what conditions chlorosis may occur from lack of iron in the leaves of the plant when an adequate supply is present in the medium. The conditions under which chlorosis may occur in the leaves as the result of iron toxicity were also investigated.

Methods

Great care was taken to expose all plants to similar environmental conditions, in so far as this was possible.

Three types of culture solutions were used. These were the modified Tottingham solution $T_1R_1C_5$ (21), as modified by JONES

TABLE I

GRAM MOLECULAR PARTIAL CONCENTRATIONS OF SALTS IN SOLUTIONS EMPLOYED

Solution	KNO_3	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	$(NH_4)_2SO_4$
Jones and Shive modified Tottingham $T_1R_1C_5$	.....	0.0021	0.0073	0.0071	0.0014
3-salt R_2S_1	.....	0.0053	0.0027	0.0132	.....
Tottingham $T_1R_1C_5$	0.002	0.0021	0.0073	0.0071	.....

and SHIVE (11), who substituted ammonium sulphate for the potassium nitrate in equivalent osmotic concentrations, the 3-salt solution R_2S_1 (18), and the Tottingham solution $T_1R_1C_5$ (21). These solutions were all used at an osmotic concentration of one atmosphere. The formulas of these three solutions are given in table I.

Two-quart colorless glass jars were used for culture receptacles, and the culture solutions were renewed continuously in these by means of "drip and drain" methods, which allowed one liter of new solution to flow into each culture jar at an approximately constant rate during a period of twenty-four hours, while during the same time an equal amount of solution, less the moisture lost by transpiration from the plants, was automatically removed from each culture jar (**19**). Each culture was continuously aerated by forcing air through the culture solutions.

Three soy bean plants were grown in each culture. The seeds had been germinated in damp sphagnum moss, and when the seedlings were about 5 cm. tall they were carefully selected for uniformity, mounted in two-piece paraffined cork stoppers, and transferred to the culture solutions.

The P_H values of the culture solutions continuously discharged from the culture jars in which the plants were growing were determined at regular intervals during the growth periods of the plants in the different experiments. The colorimetric method was used in making these tests (**3**, **7**), but the readings were occasionally checked by the electrometric method. The readings gave the record of the changes in hydrogen ion concentration of the culture solutions which had been in contact with the plant roots.

The plants of all the cultures of an experiment were harvested on the same day, and the tops and roots were dried separately in an electric oven for three days at 101° C. The dry weights of the tops and roots were then obtained by the chemical balance.

Four iron compounds, Powers-Weightman-Rosengarten's ferric glycero phosphate, soluble ferric phosphate, ferric tartrate, and ferrous sulphate were used in the culture solutions to supply iron in soluble form. A stock solution of each of these iron compounds, of such concentration that each cubic centimeter contained 0.5 mg. of iron, was freshly prepared each day just before being used in the preparation of the culture solutions.

For determining iron in solutions or in the plant tissues, the WOLTER volumetric method (**25**) was used, and the results obtained by this method were compared with the results obtained from using the WONG colorimetric method (**26**). Much time was devoted to

the trial and selection of a proper method by which to determine the iron content of the plant tissues and of the culture solutions. The gravimetric methods were eliminated because of the very small amounts of iron considered. In like manner, the potassium permanganate titration method was not employed because of the possibility of oxidation before titration could be completed. It was finally decided to make the analyses by the WOLTER method and to check these results by the WONG method, and in this way acquire confirmatory data. Both of these methods have been successfully used by the biological chemists for the determination quantitatively of exceedingly small amounts of iron in biological fluids. The apparent advantage of these methods over the other quantitative methods is their high sensitiveness, and the fact that the iron is in the stable or ferric form when measured, with no possibility of oxidation or reduction changes taking place.

Iron solubility tests

In order to determine the solubility of the four iron compounds in the several types of culture solutions, the following tests were made. Each of the solutions was adjusted to definite P_H values of 4.6, 5.5, and 6.2. To 250 cc. portions of each of the culture solutions in duplicate were added the required amounts of N/2 KOH to bring the hydrogen ion concentration of the solutions to the desired P_H values. To each 250 cc. portion of the different solutions so adjusted was then added 1 mg. of iron in the form of ferric glycero phosphate, soluble ferric phosphate, ferric tartrate, or ferrous sulphate; each form of iron being always supplied at the rate of 4 mg. of iron per liter of culture solution. These iron compounds were added singly in solution form in such concentrations that 2 cc. of the iron solution contained exactly 1 mg. of iron.

These three culture solutions, which had been adjusted to the respective P_H values and supplied with the iron compounds, were allowed to stand in the greenhouse for three days. The solutions were contained in half-liter culture jars carefully covered to protect them from the light. The precipitates, wherever these occurred, were then separated from the culture solution by filtration, and both

the filtrates and the precipitates were analyzed for their iron content by means of the WOLTER volumetric method.

In table II are given the results of the analyses of the solutions. The data represent the average actual amounts in milligrams of iron retained in the filtrates from the 250 cc. portions of the culture solutions after a period of three days, when the iron compounds were present in the solutions in concentrations of 4 mg. of iron per liter of solution.

TABLE II

AMOUNTS OF IRON IN MG. RETAINED IN FILTRATES FROM 250 CC. PORTIONS OF CULTURE SOLUTIONS

SOLUTION	P_H VALUES	AMOUNT OF IRON IN MG.			
		Ferric glycero phosphate	Soluble ferric phosphate	Ferric tartrate	Ferrous sulphate
Tottingham $T_1R_1C_5$ as modified by Jones and Shive	4.6	0.29	0.37	0.47	0.78
	5.5	0.35	0.44	0.55	0.38
	6.2	0.26	0.34	0.48	0.66
3-salt R_2S_1	4.6	0.30	0.43	0.49	0.57
	5.5	0.50	0.47	0.41	0.44
	6.2	0.29	0.17	0.37	0.13
Tottingham $T_1R_1C_5$	4.6	0.20	0.40	0.70	0.70
	5.5	0.35	0.40	0.73	0.84
	6.2	0.20	0.00	0.40	0.00

It is to be emphasized, of course, that the amounts of iron determined in the precipitates, when added to the amounts determined in the filtrates, do not always equal 1 mg., as they should if no iron had been lost in the process of analysis, but in most cases they closely approximate this value.

From the data of table II it is apparent that ferric glycero phosphate has a relatively low solubility in each of the three types of solutions, but its solubility at 4.6 and 6.2 appears to be about the same, both in the Tottingham solution and in the modified Tottingham solution. In each of the three types of solutions its solubility appears to be higher at P_H 5.5 than it is at either a lower or a higher P_H value.

Soluble ferric phosphate shows a relatively high solubility at P_H values of 4.6 and 5.5 in each of the three types of solutions, but is practically insoluble at P_H 6.2, except in the modified Tottingham solution.

Ferric tartrate has a relatively high solubility in each of the three types of culture solutions used, showing the highest solubility, however, in the Tottingham solution. It appears that the reaction of the solution, within the range of P_H values used, has no pronounced influence upon the solubility of this compound in these solutions.

Ferrous sulphate, like soluble ferric phosphate, shows relatively high solubility in each of the three types of solutions at P_H values of 4.6 and 5.5, but is insoluble at P_H values of 6.2, except in the modified Tottingham solution, where it shows relatively high solubility.

Assuming that the availability to the plants of iron in a culture solution is closely correlated with the solubility of the iron compound in the solution, the data here considered suggest in a general way that ferric glycero phosphate could be used to advantage as a source of iron for plants, in culture solutions in which the hydrogen ion concentration is maintained at a high level during contact with the plant roots without much danger of iron toxicity, which is quite likely to occur in plants grown in a culture solution of this type when soluble ferric phosphate or ferrous sulphate is used as a source of iron. It is to be expected that soluble ferric phosphate and ferrous sulphate can have little efficiency as sources of iron for plants in culture solutions with P_H values approaching the neutral point. In solutions of this type, ferric tartrate should prove more effective as a source of iron for plants than any other of the iron compounds here considered.

Experiments

EXPERIMENT 1.—This was conducted to obtain, if possible, some experimental evidence as to the conditions under which a high or a low absorption of iron by the plant might take place.

In devising this experiment, it was planned to use different iron compounds in the same culture solution, and also to use the same iron compound in different culture solutions. Eighteen cultures were used and were carried out in duplicate. The cultures were constantly aerated and the culture solution continuously renewed. The experiment was conducted during a period of nine weeks, but before harvesting the plants they were carefully examined for degrees of chlorosis and general appearance with respect to health, vigor, etc. Three series of cultures were included in the experiment, designated as series A, B, and C, according as ferric

glycero phosphate, soluble ferric phosphate, or ferrous sulphate was supplied to the cultures as the source of iron for the plants. The iron compounds in solution form were added to the culture solutions to supply equal amounts of iron per culture. The iron concentrations in milligrams per liter of culture solution for the different intervals during the growth period are given in the footnote of table III.

Iron analyses were made on the total tops of the three plants comprised in each culture. The percentage of iron in the tops was calculated on the dry weight basis. The dry weights of the roots and the yields of iron in the roots, although determined in many cases, were not considered reliable, since it was found that a slight precipitate containing iron had collected on the outside of the roots, which could not readily be removed, in this way producing too high percentages of iron for the roots. There was great variability in the amounts of iron in the roots, and little agreement between the iron contents of the roots of duplicate cultures.

The average P_H values of discharged culture solution during the growth period, the condition of the plants, the dry weights of tops, and the percentage of iron in tops of plants grown in duplicate cultures in series A, B, and C are given in table III. From these data, several interesting facts may be observed. In general, it may be said that high yields of tops correspond to low yields of iron in the plant tops. As a rule, the cultures in which the plants gave no visible indication of toxic effects and were entirely free from chlorosis produced the highest dry weight yields in the respective series.

In the comparison of the plants there were two types of chlorosis easily distinguishable. Plants exhibiting the first type bore pale yellow leaves. This chlorotic condition is characteristic of plants suffering from lack of iron (13), and occurs first in the newly formed leaves. The leaves of plants exhibiting the second type did not present the characteristic yellow appearance from lack of iron, but the older leaves were covered with a yellow and green mottling. Many of the plants which were growing in the modified Tottingham solution exhibited a peculiar brown specking, especially on the unfolding new leaves This condition was later succeeded by a general mottling of the leaves. All the plants showing the toxic

condition presented an unhealthy appearance, with mottled, dying, or dead leaves (**13**).

TABLE III

AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHT YIELDS, AND PERCENTAGE OF IRON IN TOPS OF PLANTS OF SERIES A, B, AND C OF EXPERIMENT I*

Culture solution	Average P_H	Condition	Dry weight tops, three plants (gm.)	Percentage iron in tops
	A: ferric glycero phosphate			
Modified Tottingham	4.60	Normal	8.790	0.025
	4.50	Normal	10.100	0.019
Average	4.55		9.445	0.022
3-salt R_2S_1	5.00	Chlorotic	5.880	0.052
	5.00	Chlorotic	4.420	0.056
Average	5.00		5.150	0.054
Tottingham $T_1R_1C_5$	5.00	Chlorotic	6.120	0.068
	5.50	Chlorotic	5.220	0.069
Average	5.25		5.670	0.068
	B: soluble ferric phosphate			
Modified Tottingham	4.70	Toxic	5.590	0.073
	4.80	Toxic	4.540	0.097
Average	4.75		5.056	0.085
3-salt R_2S_1	5.10	Normal	22.590	0.007
	5.10	Normal	24.250	0.009
Average	5.10		23.420	0.008
Tottingham $T_1R_1C_5$	5.20	Normal	11.380	0.023
	5.20	Normal	15.070	0.022
Average	5.20		13.225	0.022
	C: ferrous sulphate			
Modified Tottingham	4.70	Toxic	7.850	0.0500
	4.40	Toxic	9.520	0.0460
Average	4.55		8.685	0.0480
3-salt R_2S_1	5.20	Normal	9.420	0.0410
	5.20	Normal	10.780	0.0420
Average	5.20		10.100	0.0415
Tottingham $T_1R_1C_5$	4.80	Normal	8.950	0.0390
	4.90	Normal	8.560	0.0420
Average	4.85		8.755	0.0405

* Iron additions per liter of culture solution: January 17–29, 0.1 mg.; January 30–31, 0.3 mg.; February 1–12, 0.5 mg.; February 13–19, 1 mg.; February 20–March 16, 2 mg.; total iron added per culture during growth period, 64.9 mg.

In table III this second type of chlorosis is indicated as the toxic condition of the plants. The general tendency shown by the plants of these series, as indicated in this table, was that the plants possessing either the chlorotic or the toxic condition have a higher total percentage of iron than do the normal plants. It may be seen that all the plants showing the toxic condition are to be found in the modified Tottingham solution, the average P_H values of which, after being in contact with the plant roots, are indicated in table III as being relatively very low. This condition of the plants when grown in the modified Tottingham solution has been pointed out by JONES and SHIVE (13), who explain the phenomena by suggesting that the presence of ammonium sulphate in a solution, either through its influence on the hydrogen ion concentration of the culture solution in contact with growing plant roots, or the possible effect it may have on cell permeability makes the iron more available, and may account for its toxic effects.

It appears from the data obtained that, in normal growth, activities progress most favorably when the plants contain only a relatively low percentage of iron. The condition in which the iron is present within the plant is also possibly very important. A small amount of mobile iron may become equally distributed in roots, stems, petioles, and leaves, and act efficiently; while a larger amount may either lodge in some portion of the roots, stems, or petioles, or perhaps be distributed in such concentration as to result in retarding development. This, of course, is a question involving many complex phenomena, among which must be considered the relative solubilities of the various iron compounds and the compounds of iron formed within the plant (23). In addition to the condition of the iron compounds, consideration must also be given to the changes taking place in the culture media in which the plants are grown, and the effects produced within the plant by contact with the changing culture media. All these are influenced by the variable environmental complex, which, of course, exert an influence on the efficiency of the iron content.

It may be concluded that relatively very small quantities of iron within the plant promote its normal development. It is observed, however, that the nature of the culture solution determines, in a large measure, the rates of iron absorption, since it has been

shown that the toxic condition occurs in the plants grown in the culture solution containing ammonium sulphate. No chlorosis, however, occurred in these plants as a result of lack of iron; on the other hand, chlorosis resulting from lack of iron occurred in some of the plants grown in the three-salt solution and in the Tottingham solution, and always in these solutions when employed with ferric glycero phosphate as the source of iron.

EXPERIMENT II.—This experiment, like the one preceding, was conducted with the object of determining under what conditions a high or a low absorption of iron may take place. The reason for repeating the experiment was primarily to confirm by further observation the apparently contradictory evidence of the preceding experiment, that plants chlorotic from lack of iron in the leaves, as well as those showing the toxic condition, possessed a higher iron content than do the normally healthy plants.

The method of procedure was similar to that employed in experiment I. The same three types of culture solutions and the same three iron compounds were again employed. The plants were five weeks old when harvested. The numerical data of experiment II are presented in table IV.

Here it is again shown that high yields of tops correspond to low yields of iron in the plants. In general, the dry weight yields of the tops are lower than the dry weight yields shown for the preceding experiment, and the percentages of iron in the tops throughout each series are considerably higher than are those shown for the preceding experiment. The relationships which were observed in the preceding experiment, however, are found to be almost identical with those in the present experiment. The lower yields and the higher percentages of iron shown for the tops in the present experiment may be accounted for by the fact that the data in table III refer to plants nine weeks old, while the data in table IV refer to plants only five weeks old.

As before, there were two types of chlorosis easily distinguishable. The mottled yellow, or toxic type, always occurred in the modified Tottingham solution, and this solution, after being in contact with the roots, possessed a relatively high hydrogen ion concentration during the growth period. No chlorotic plants of the pale yellow type were found in the modified Tottingham solution in

either experiment I or II. The chlorotic plants were to be found distributed in the three-salt and Tottingham solutions.

TABLE IV

AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHT YIELDS, AND PERCENTAGE OF IRON IN TOPS OF PLANTS OF SERIES A, B, AND C OF EXPERIMENT II*

Culture solution	Average P_H	Condition	Dry weight tops, three plants (gm.)	Percentage iron in tops
	A: ferric glycero phosphate			
Modified Tottingham	4.60	Normal	6.260	0.0380
	4.50	Normal	7.200	0.0470
Average	4.55		6.730	0.0425
3-salt R_2S_1	5.30	Chlorotic	1.970	0.0810
	5.30	Chlorotic	2.300	0.0800
Average	5.30		2.153	0.0805
Tottingham $T_1R_1C_5$	5.30	Chlorotic	2.870	0.0700
	5.30	Chlorotic	3.610	0.0600
Average	5.30		3.240	0.0650
	B: soluble ferric phosphate			
Modified Tottingham	4.90	Toxic	4.000	0.0730
	4.90	Toxic	4.160	0.0620
Average	4.90		4.800	0.0675
3-salt R_2S_1	5.40	Chlorotic	5.320	0.0710
	5.40	Chlorotic	4.900	0.0600
Average	5.40		5.110	0.0655
Tottingham $T_1R_1C_5$	5.60	Normal	6.190	0.0330
	5.60	Normal	5.220	0.0550
Average	5.60		5.705	0.0440
	C: ferrous sulphate			
Modified Tottingham	4.60	Toxic	4.010	0.1000
	4.60	Toxic	3.140	0.1400
Average	4.60		3.575	0.1200
3-salt R_2S_1	5.40	Chlorotic	5.150	0.0420
	5.30	Chlorotic	4.790	0.0330
Average	5.35		4.970	0.0375
Tottingham $T_1R_1C_5$	5.50	Chlorotic	5.450	0.1500
	5.50	Chlorotic	5.600	0.0530
Average	5.50		5.525	0.1015

* Iron additions per liter of culture solutions: March 14–21, 0.1 mg.; March 22–26, 0.5 mg.; March 27–31, 1 mg.; April 1–4, 2 mg.; April 5–10, 1 mg.; April 11–16, 2 mg.; total iron added per culture during growth period, 36.3 mg.

As in the preceding experiment, so from the data of this experiment also it appears that small percentages of iron favor the development of a normal healthy condition. The condition in relation to the iron constituents, of course, is influenced by the variable complex of the environment. That relatively small quantities of iron within the plant promote its normal development, and that higher concentrations of iron are not conducive to normal growth may be concluded from the data of the two experiments considered. It is important to note that there is a very narrow range of iron concentration in the culture solutions, under a given set of experimental conditions, which will maintain the plants in a healthy, green condition. A concentration of iron in the medium, either slightly above or below that required to maintain the plants in the normally green condition, will produce either the toxic or the chlorotic conditions noted.

The anomalous condition with respect to the abnormally high iron content in the tops showing both the toxic and the chlorotic condition, which appears repeatedly in the data presented in tables III and IV, will be considered in connection with the data of the following experiment, which was undertaken mainly in an endeavor to account for and to clear up this apparently contradictory evidence.

EXPERIMENT III.—This experiment was conducted to determine, if possible, the distribution of the iron in the tops when the plants had been grown under conditions similar to those of the two preceding experiments.

The modified Tottingham solution, the three-salt solution, and the Tottingham solution were used as before. Ferric glycero phosphate, soluble ferric phosphate, ferric tartrate, and ferrous sulphate were employed as the sources of iron. The amounts of iron per liter supplied to the solutions during the different intervals are shown in the footnote of table V. The cultures were conducted in duplicate. The plants were grown through a period of thirty-five days, and when harvested the leaves were separated from the stems in each culture and the dry weights of stems and leaves determined separately. After the dry weights were obtained, the leaves and the stems were analyzed separately for their iron content, and the percentages of iron were calculated on the dry weight basis. The numerical data of the experiment are presented in table V.

TABLE V

AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHTS OF LEAVES AND STEMS, AND PERCENTAGE OF IRON IN STEMS AND LEAVES OF PLANTS OF SERIES A, B, C, AND D OF EXPERIMENT III*

SERIES	AVERAGE P_H	CONDITION	DRY WEIGHTS (GM.)			PERCENTAGE IRON		
			Leaves	Stems	Entire tops	Leaves	Stems	Entire tops
	Modified Tottingham solution $T_1R_1C_5$							
A: Ferric glycero phosphate	4.15	Normal	4.48	6.50	10.98	0.0100	0.0160	0.0120
	4.15	Normal	4.87	5.03	9.90	0.0090	0.0150	0.0120
Average	4.15		4.67	5.26	10.44	0.0095	0.0155	0.0120
B: Soluble ferric phosphate	4.25	Normal	5.09	4.67	9.76	0.0100	0.0150	0.0120
	4.25	Normal	4.11	3.51	7.62	0.0080	0.0200	0.0140
Average	4.25		4.60	4.09	8.69	0.0090	0.0180	0.0130
C: Ferric tartrate	4.30	Toxic	4.65	4.29	8.94	0.0140	0.0260	0.0190
	4.25	Toxic	3.36	2.82	6.16	0.0170	0.0560	0.0340
Average	4.27		4.00	3.55	7.55	0.0155	0.0410	0.0265
D: Ferrous sulphate	4.36	Toxic	1.85	3.01	4.86	0.0210	0.0670	0.0490
	4.45	Toxic	1.70	1.33	3.03	0.0350	0.1160	0.0750
Average	4.40		1.77	2.17	3.94	0.0280	0.0900	0.0620

* Iron additions per liter of culture solution: May 23–June 3, 0.2 mg.; June 4–5, 0.5 mg.; June 6–24, 1 mg.; total iron added per culture during growth period, 20.9 mg.

	3-salt solution R_2S_1							
A: Ferric glycero phosphate	5.03	Chlorotic	2.97	1.21	4.18	0.0080	0.0800	0.0260
	5.06	Chlorotic	4.93	1.41	6.34	0.0040	0.0690	0.0180
Average	5.04		3.96	1.31	5.26	0.0060	0.0740	0.0220
B: Soluble ferric phosphate	5.43	Normal	4.81	4.78	9.59	0.0160	0.0190	0.0170
	5.16	Normal	4.39	4.09	8.48	0.0200	0.0150	0.0170
Average	5.29		4.60	4.43	9.03	0.0180	0.0170	0.0170
C: Ferric tartrate	5.36	Slightly	3.30	4.36	7.66	0.0170	0.0200	0.0180
	5.36	chlorotic	3.49	3.01	6.50	0.0160	0.0280	0.0210
Average	5.36		3.39	3.68	7.07	0.0165	0.0240	0.0190
D: Ferrous sulphate	5.43	Normal	5.53	5.10	10.63	0.0130	0.0090	0.0110
	5.26	Normal	5.22	5.32	10.54	0.0130	0.0140	0.0130
Average	5.34		5.37	5.21	10.58	0.0130	0.0115	0.0120
	Tottingham solution $T_1R_1C_3$							
A: Ferric glycero phosphate	5.50	Slightly	4.67	4.60	9.27	0.0140	0.0150	0.0140
	5.30	chlorotic	4.50	5.10	9.60	0.0100	0.0130	0.0110
Average	5.40		4.58	4.85	9.43	0.0120	0.0140	0.0125
B: Soluble ferric phosphate	5.66	Slightly	4.87	4.54	9.41	0.0090	0.0210	0.0140
	5.76	chlorotic	3.96	4.45	8.41	0.0130	0.0320	0.0200
Average	5.71		4.41	4.49	8.90	0.0110	0.0270	0.0170
C: Ferric tartrate	5.73	Slightly	4.58	4.88	4.46	0.0090	0.0250	0.0170
	5.83	chlorotic	4.70	5.18	9.88	0.0080	0.0290	0.0190
Average	5.78		4.64	5.03	9.67	0.0085	0.0270	0.0180
D: Ferrous sulphate	5.80	Slightly	4.78	4.84	9.62	0.0130	0.0210	0.0170
	5.80	chlorotic	4.93	4.69	9.62	0.0170	0.0290	0.0240
Average	5.80		4.85	4.76	9.62	0.0150	0.0250	0.0205

It is here again shown that high yields of tops correspond to low yields of iron, but it is further shown that the iron is fairly uniformly distributed in the stems and leaves of the normally healthy plants. These plants usually produced the highest dry weight yields of tops and showed the lowest iron content for whole tops in the respective series. The plants showing the toxic condition are always to be found in the modified Tottingham solution, and in these the iron content is unevenly distributed, being higher in the stems than in the leaves. It is, however, usually higher in both the stems and leaves than in the corresponding parts of normally healthy plants. The chlorotic plants show a lower concentration of iron in the leaves and a much higher concentration of iron in the stems than do the normally healthy plants. Even the slightly chlorotic plants generally have a higher iron content in the stems than in the leaves. All the chlorotic plants, as well as all the plants showing the toxic condition, have as high or a higher total iron content than do the normal plants. The explanation of the apparent anomaly previously referred to is here made clear. The data of table V show that the abnormally high iron content for entire tops of the chlorotic plants may be accounted for by the fact that exceedingly high concentrations of this element are found in the stem, but relatively very low concentrations in the leaves. It thus appears that very low concentration of iron in the leaves accompanied by abnormally high concentrations of this element in the stems results in the chlorotic conditions observed, since only very small percentages of iron finally reach the leaves, the larger portion of the total iron being lodged in roots, stems, or petioles, and since the small amounts finally reaching the leaves may be by no means uniformly distributed even in these organs. The chlorotic condition resulting from lack of iron in the leaves may thus occur with an abundance of iron in the plant. The plants showing the toxic condition invariably have a high iron concentration throughout, although this is by no means uniformly distributed in stems and leaves.

It is apparent that a small percentage of iron equally distributed throughout the plant promotes its health and vigor. This condition is closely related to the proper adjustment of the available iron

supply in the culture medium. If, however, available iron is present in the culture solutions in relative large amounts, and is absorbed by the plant in excess of that required to maintain a healthy green condition, some of this iron may be precipitated in mass within the roots, stems, or petioles before reaching the leaves, and the leaves may actually become chlorotic because they lack the required concentration of active iron to accompany the process of chlorophyll formation. It appears that this is what actually does occur, since the data of table V show relatively low iron content in the leaves and high iron content in the stems of chlorotic plants.

The solubility of the iron absorbed, of course, may be closely related to the hydrogen ion concentration of the plant juices, a point which has not yet received adequate attention.

These data not only emphasize the conclusions previously reached, but also show that the range in concentration of available iron in the medium, as well as the range of concentration of this element within the plant which will promote vigorous growth and favor the development of healthy green leaves, is very narrow. Concentrations of available iron in the medium, or concentrations of this element within the plant which are very slightly above or below the narrow optimum range tend to produce either the toxic or the chlorotic condition, and these same phenomena are likely to occur when the iron absorbed, even in quantities within the optimum range, is for some reason not fairly uniformly distributed throughout the plant.

EXPERIMENT IV.—The purpose of this experiment was to determine whether the iron supply could be so adjusted that normal plants might be grown in each of the three different solution types with any one of the iron compounds employed as the source of iron for the plants.

Soy bean plants of the Manchu variety were grown as before in constantly aerated and continuously renewed solutions, and were harvested at the end of an experimental period of five weeks. The iron supplies were adjusted, as nearly as could be determined, to meet the requirements of the plants. The appearance of the plants in each culture was used as an index of the amount of iron to be supplied in each case. This iron adjustment was not practiced

in the preceding experiments, but each culture of the experiment was given a definite and like amount.

Table VI presents the numerical data of this experiment. The amounts of iron supplied to each culture solution from day to day are omitted from this table but are given in table VII. Failure,

TABLE VI

AVERAGE P_H VALUES OF DISCHARGED SOLUTIONS, DRY WEIGHT YIELDS, AND PERCENTAGE OF IRON IN TOPS OF PLANTS OF SERIES A, B, C, AND D OF EXPERIMENT IV

Solution	Average P_H	Condition	Dry weight tops, three plants (gm.)	Percentage iron in tops
	A: ferric glycero phosphate			
Modified Tottingham $T_1R_1C_5$	4.20	Normal	24.20	0.016
3-salt R_2S_1	6.06	Normal	35.20	0.020
Tottingham $T_1R_1C_5$	6.40	Normal	23.98	0.018
	B: soluble ferric phosphate			
Modified Tottingham $T_1R_1C_5$	4.16	Normal	20.15	0.017
3-salt R_2S_1	6.10	Normal	28.47	0.020
Tottingham $T_1R_1C_5$	6.60	Normal	31.07	0.017
	C: ferric tartrate			
Modified Tottingham $T_1R_1C_5$	4.13	Normal	30.82	0.020
3-salt R_2S_1	6.10	Normal	26.14	0.014
Tottingham $T_1R_1C_5$	6.60	Normal	26.17	0.018
	D: ferrous sulphate			
Modified Tottingham $T_1R_1C_5$	4.16	Normal	17.00	0.017
3-salt R_2S_1	6.00	Normal	27.37	0.012
Tottingham $T_1R_1C_5$	6.63	Normal	35.28	0.016

in some instances, to record the amounts of iron in the form of ferric tartrate and ferrous sulphate added to the modified Tottingham solution, makes the data for these two forms of iron in this solution incomplete, and for this reason they have been omitted from the table.

It is again shown in table VI that the high yields of tops correspond to relatively low iron content. These cultures produced

plants which were free from chlorosis or the toxic condition, and also gave high dry weight yields.

The plants were all very large, dark green, and in every respect gave the appearance of health and vigor. There was a marked degree of uniformity in size and general appearance, regardless of the solution in which they were grown or the compound which furnished the source of iron. It should be noted, however, that the total yield from all the cultures with a single solution type is highest for the three-salt solution, only very slightly inferior for the Tottingham solution, but somewhat lower for the modified Tottingham solution.

The same marked uniformity of the iron content throughout the different series may be observed as indicated for yields, regardless of the marked differences in the hydrogen ion concentration of the discharged solution.

The reason for the marked uniformity in the data here considered and the concomitant healthy vigor of the plants may be explained if table VII is consulted and the daily fluctuations in iron increments are considered. The condition of the plants was constantly observed, and their appearance used as an index of the quantity of iron to be supplied in each case. For example, if the unfolding leaves on any particular day assumed a very faint yellow appearance, the supply of iron was slightly but immediately increased in the culture solution. On the other hand, if the plants on any particular day showed a slight lack of luster in the unfolding leaves, a curled margin of these leaves, or a faint indication of the characteristic mottled appearance in the older leaves, then the iron supply was immediately reduced in quantity, or altogether eliminated for a period. Table VII shows that relatively very small quantities of iron were required in the modified Tottingham solution to maintain the plants in a healthy, vigorous, green condition, but the percentage of iron in the plants grown in this solution is not markedly different from that of those grown in the three-salt or the Tottingham solution. This indicates that the iron supply in this solution containing ammonium sulphate is more available than is that in the other solution types, or that the plants in this solution possess a higher capacity for iron absorption by reason of

TABLE VII

CONCENTRATION OF IRON IN MG. PER LITER OF CULTURE SOLUTION FOR VARIOUS INTERVALS THROUGHOUT GROWTH PERIOD OF EXPERIMENT IV

INTERVALS	CULTURE SOLUTIONS									
	Modified Tottingham solution $T_1R_1C_2$ (mg.)		3-salt R_2S_1 (mg.)				Tottingham $T_1R_1C_2$ (mg.)			
	F.G.P.*	S.F.P.	F.G.P.	S.F.P.	F.T.	F.S.	F.G.P.	S.F.P.	F.T.	F.S.
June 27–28	0.10	0.05	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
29–30	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
July 1–2	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.1
3	0.20	0.10	0.20	0.10	0.10	0.10	0.20	0.10	0.10	0.1
4	0.20	0.10	0.20	0.20	0.20	0.10	0.20	0.20	0.20	0.2
5–6	0.30	0.10	0.20	0.20	0.20	0.10	0.20	0.20	0.20	0.1
7–8	0.20	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.1
9–10	0.20	0.10	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.2
11–12	0.40	0.10	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.4
13	0.20	0.10	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.4
14	0.20	0.20	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.6
15	0.20	0.10	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.6
16	0.20	0.00	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.6
17	0.20	0.05	0.60	0.60	0.60	0.60	0.60	0.60	0.60	0.6
18	0.20	0.10	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.8
19–21	0.40	0.10	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.8
22–23	0.40	0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.0
24–26	0.00	0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.0
27	0.80	0.20	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
28	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
29–30	0.00	0.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.0
31	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.0
August 1–3	0.40	0.20	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.0
Total	9.70	4.05	20.10	19.80	19.20	19.40	20.00	19.80	19.70	19.4

* F.G.P. = ferric glycero phosphate; S.F.P. = soluble ferric phosphate; F.T. = ferric tartrate; F.S. = ferrous sulphate.

greater permeability of the cell membranes involved in the absorption of this element, as has been suggested by JONES and SHIVE (13). This suggestion is supported by the fact that when the iron concentration in this culture solution was relatively high, the iron content was correspondingly high, as shown in preceding experiments, and these plants frequently showed the unhealthy toxic condition caused by an abnormally high iron content throughout the plant. This condition could not occur in the cultures of the present experiment, because the supply of soluble iron was added to the culture solution only as the plants, by their appearance, seemed to require it. In this way, both iron toxicity and chlorosis were prevented. It is to be suggested, however, that the maintenance of the high hydrogen ion concentration in the modified Tottingham solution (by contact with the roots), as indicated by the P_H values of the discharged solution, may account in a large measure for the high availability of the iron supply in this solution.

It thus appears that when the available iron supply in the medium is properly adjusted to meet the requirements of the plants, any one of the culture solutions here employed, supplied with any one of the compounds used as a source of iron, may be expected to produce healthy, vigorous growth of soy bean plants under cultural conditions similar to those here applied.

Discussion

In order to bring out more clearly the relation between iron content and the conditions of the plants (normal, chlorotic, or toxic) as these have been determined by observation and by methods of comparison, the numerical data of the plants showing these conditions have been brought together from tables III, IV, and V, and are shown diagrammatically in figs. 1 and 2. Fig. 1 represents the data brought together from tables III and IV. The data referring to the percentages of iron in the tops and the total dry weights of all plants showing the normal healthy condition were averaged, and the same was done for all plants showing the toxic and chlorotic condition, and from these average data the diagram of fig. 1 was constructed. It is to be emphasized that the data of the figures are the average values for plants ranging in numbers from a minimum of approximately 36 to a maximum of about 100.

Fig. 1 shows that the average percentage of iron in the tops of all the healthy, vigorous plants possessing normally green leaves was only about one-half as high as it was for plants showing the chlorotic condition characteristic of too little iron in the leaves, and considerably less than one-half as high as that for plants showing the toxic condition resulting from too much iron in the leaves.

The abnormally high average iron content shown for plants having leaves chlorotic from lack of iron, as previously explained, apparently results from the lodgment of iron in the stems and per-

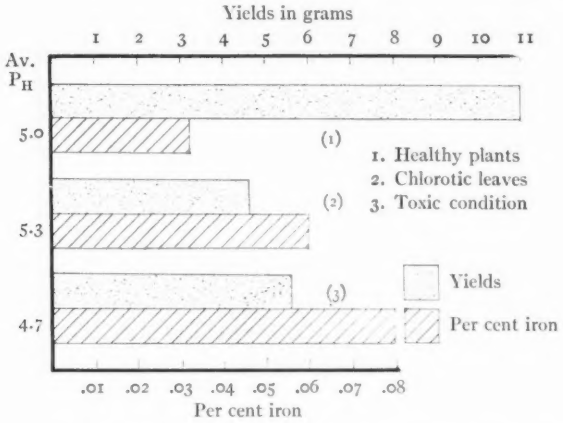

FIG. 1.—Yields and percentage of iron in tops of healthy plants, and in those showing chlorotic or toxic condition.

haps in the roots also, before a supply sufficient to maintain a green healthy color reaches the leaves (fig. 2).

The influence of the chlorotic and toxic conditions of the plants upon growth rates and yields is quite marked (fig. 1). This diagram indicates that the average yield of tops from the cultures producing the chlorotic plants and from those producing plants showing the toxic condition is less than one-half and about one-half, respectively, of the corresponding yield from the cultures which produced healthy, vigorous, normally green plants.

Fig. 2 represents the average data brought together from table V, but the average percentages of iron in stems and leaves are

represented separately. The relations brought out by this diagram, in general, are the same as are those shown by fig. 1. The average percentages of iron for entire tops are much higher for plants showing the chlorotic or the toxic condition than is the corresponding percentage of iron in healthy plants.

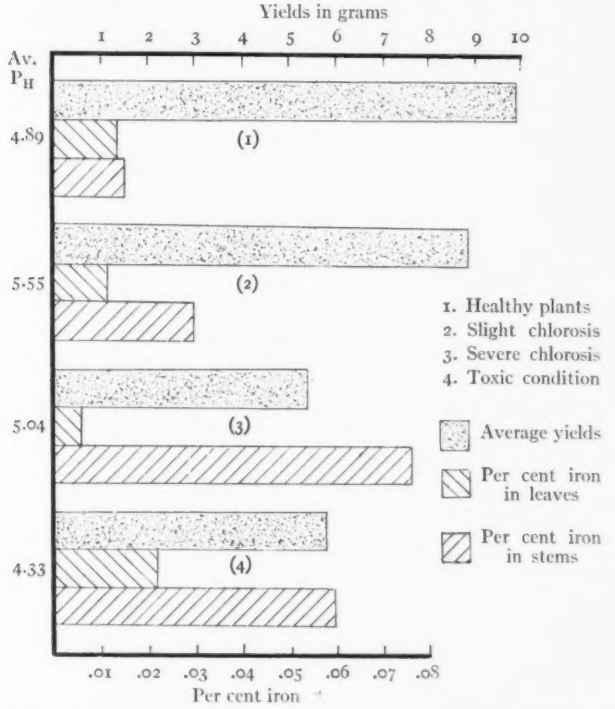

FIG. 2.—Average yields and percentage of iron in leaves and stems of healthy plants, and in leaves and stems of plants showing chlorotic or toxic condition.

It will be observed that the average percentage of iron in healthy plants is relatively quite low in both stems and leaves, and is fairly uniformly distributed. It is to be noted, however, that the average percentage of iron in the leaves of plants showing the marked chlorotic condition is very much lower than is the corresponding percentage of iron in leaves of healthy plants. On the other hand,

the average percentages of iron in the stems of plants showing the chlorotic and the toxic condition are nearly twice as high and five times as high, respectively, as is the corresponding percentage of iron in the stems of healthy plants. This indicates that, although under certain conditions plants may contain an abundant supply of iron, they may still show the characteristic chlorotic conditions of leaves suffering from lack of iron, owing to the fact that the iron is not properly distributed.

At the present time no definite suggestion can be offered with respect to the probable causes of the unequal distribution of iron in these plants, nor why this element should become concentrated in the stems and perhaps in the roots before reaching the leaves. It is possible, however, that this phenomenon may be directly related to the reaction change which the plants themselves may bring about in the medium in which they were grown. For example, it will be observed (fig. 2) that the average P_H value (determined at frequent intervals throughout the growth period) of the solutions continuously discharged from the cultures which produced the normally healthy plants varies only slightly from the corresponding initial P_H value of these culture solutions. On the other hand, the average P_H values of the discharged solution which had been in contact with the roots of plants showing the chlorotic condition, and with those of plants showing the toxic condition, were much higher and somewhat lower, respectively, than were the corresponding initial P_H values.

It appears from the foregoing considerations that a small percentage of iron uniformly distributed is requisite for a state of health in the plant. Excessive amounts of iron may either lodge in some portion of the roots, stems, or petioles, or perhaps may be distributed throughout the plant in such concentration as to become toxic. The condition in which the iron is present is undoubtedly also very important. A small amount of mobile iron may become equally distributed in roots, stems, petioles, and leaves, and may act efficiently in the metabolic process or as a catalytic agent in chlorophyll formation.

In making a comparison of the relative efficiency of different culture solutions, it is necessary to take into consideration an

adjustment of the iron supply to the requirements of the plants growing in the culture. Unless the iron supply is properly adjusted, a culture solution will usually not produce good plants.

Summary

1. A study was made of the effects of four soluble iron compounds, used singly, upon the growth and appearance of soy bean plants grown in three types of culture solutions, continuously renewed and constantly aerated.

2. When the iron supply was adjusted from day to day to meet the requirements, large, healthy, vigorous plants were produced, regardless of the type of culture solution or the iron compound employed.

3. When the available iron supply in the medium is slightly in excess of that actually required, the plants may become chlorotic from failure of iron to reach the leaves in the necessary concentration, or they may show the symptoms characteristic of iron toxicity.

4. Quantitative analyses for iron show that healthy green plants possess lower average percentages of iron, on the dry weight basis, than do chlorotic plants or those suffering from iron toxicity. The iron in healthy green plants, however, although relatively low in concentration, appears to be uniformly distributed throughout the stems and leaves, while the iron content of the chlorotic plants is high in the stems and very low in the leaves, and that of the plants suffering from iron toxicity is high throughout.

5. In order to maintain the plants in the healthy green condition, the supply of soluble iron in the culture solution must be maintained at as low a concentration as possible without inducing chlorosis from lack of available iron. A concentration of soluble iron slightly above this optimum may result in iron toxicity from a high content throughout the plant, or chlorosis may occur due to the lodgment of iron in the roots and stems, thus preventing the distribution of the element to the leaves in amounts sufficient to provide adequately for proper chlorophyll formation.

6. Small additions of available iron to the culture solution as the plant appears to require it tend to produce equal distribution throughout, and to keep it in a healthy green condition. The

general appearance of the plant must serve as an index of the iron supply in each case. Definite applications of iron to the culture media at fixed intervals during any given physiological stage of development is not practicable, since the ever changing plant environment has a pronounced influence upon the iron requirement.

7. It appears that a delicate balance exists within the plant which requires that the available iron in the culture medium be limited to a very narrow range of concentrations to produce optimum growth.

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A DISTORTION OF THE 3:1 RATIO

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 329

MERLE C. COULTER

Discovery of abnormal ratios

In connection with some experiments conducted in 1919, an ear of corn (710) was produced by self-fertilizing an individual heterozygous for the C factor. As expected, a ratio of 3 colored : 1 white appeared among the grains of this ear. It was further expected that two-thirds of these colored grains would be heterozygous and yield normal 3:1 ratios in the following generation. For the most part, this was the result actually obtained, but three of them gave decided departures from the 3:1 ratio (table I).

TABLE I

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
939.....	11.6	191	25	4.29	29.0	6.76	300,000:1
937.....	11.0	210	26	4.49	33.0	7.35	Over 1,000,000:1
938.....	10.0	207	23	4.43	34.5	7.79	Over 1,000,000:1

Such deviation from the normal ratio was of undoubted significance. Whether this was a genetic significance could be determined by further breeding. The following season, therefore, all of the colored grains of 938 were planted, and a few of 939. As a check, some of the grains of 940 were planted; this was a sib which had shown the normal 3:1 ratio. For the most part, the plants of the next generation were selfed. The results of these selfings appear in tables II, III, and IV.

The progeny of 940 satisfied the expectations, in that exactly one-third of the plants proved to be homozygous, and two-thirds heterozygous for C. The six ratios obtained, however, are perhaps not all as close to the perfect 3:1 as had been expected from previous experience. I believe that the ratio of 140:31 which appears on ear 193 is actually a 3:1 ratio, but the chances are only about one out of 26 that this is the case. Similarly the chances are one out of 13 that

the ratio of ear 195 is a 3:1. The remaining four are normal ratios. In any event, it is clear that the progeny of 940 show nothing like the decided departures from the 3:1 that are in evidence among the progeny of 939 and 938.

The data provided by the progeny of 939 and 938 are apparently quite similar. In the former only a few grains were planted, and

TABLE II

RESULTS OF SELFING PLANTS OF 940 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
198	0.0	150	0	.....	.....	.....	.....
192	0.0	300	0	.....	.....	.....	.....
191	0.0	150	0	.....	.....	.....	.....
193	18.1	140	31	3.82	11.75	3.07	25:1
197	22.3	73	21	2.83	2.50	Under 1	1:1
190	24.3	134	43	3.89	1.25	Under 1	1:1
196	24.6	203	66	4.79	1.25	Under 1	1:1
194	25.0	111	37	3.57	0.00	Under 1	1:1
195	30.3	145	63	4.21	11.00	2.61	12:1

TABLE III

RESULTS OF SELFING PLANTS OF 939 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
154	0.00	10	0	.....	.....	.....	.....
152	6.8	55	4	2.24	10.75	4.8	825:1
153	12.5	42	6	2.02	6.00	3.0	22:1
151	12.5	21	3	1.43	3.00	2.1	5:1
150	14.3	18	3	1.34	2.25	1.7	3:1
156	27.3	32	12	1.94	1.00	Under 1	1:1
155	33.3	22	11	1.68	2.75	1.6	3:1

the resulting plants were relatively weak, so that no large ears were obtained. Yet this limited population reveals the same characteristics as does the much larger population of 938, and doubtless the same would have been true of 937.

It is quite evident that the condition which results in deficiency of white grains is heritable. Not that every resulting ear shows such deficiency, for there are clearly some that give all colored and others that give the normal 3:1 ratio; but there are many ears in which an undoubted deficiency of the white grains appears. As for the variation in the amount of white deficiency, that is a secondary problem which will be mentioned later.

TABLE IV

RESULTS OF SELFING PLANTS OF 938 FAMILY (29 EARS GAVE ALL COLORED)

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
119	7.5	37	3	1.85	7.00	3.78	92:1
466	8.2	201	18	4.32	36.75	8.51	10,000,000,000:1
433	8.6	96	9	2.99	17.25	5.77	10,000:1
429	10.3	253	29	4.90	41.50	8.47	10,000,000,000:1
122	10.6	185	22	4.20	29.75	7.08	500,000:1
417	10.9	221	27	4.60	35.00	7.61	10,000,000:1
445	11.2	230	29	4.70	35.75	7.61	10,000,000:1
427	11.7	234	31	4.75	35.25	7.42	2,000,000:1
410	12.0	243	33	4.85	36.00	7.42	2,000,000:1
419	12.0	257	35	4.99	38.00	7.61	10,000,000:1
412	12.2	238	33	4.81	34.75	7.22	1,000,000:1
118	12.5	140	20	3.69	20.00	5.42	5,000:1
110	12.8	198	29	4.40	27.75	6.31	50,000:1
120	13.3	111	17	3.30	15.00	4.55	450:1
431	13.9	182	27	4.22	25.25	5.99	20,000:1
425	14.5	65	11	2.55	8.00	3.14	28:1
422	14.6	169	29	4.11	20.50	4.99	1,300:1
447	15.3	198	36	4.47	22.50	5.03	1,400:1
463	16.0	199	38	4.50	21.25	4.72	700:1
401	16.2	223	43	4.76	23.50	4.94	1,250:1
420	16.5	66	13	2.60	6.75	2.60	12:1
461	16.7	233	47	4.89	23.00	4.70	600:1
440	17.0	83	17	2.92	8.00	2.74	14:1
415	17.1	199	41	4.52	19.00	4.20	220:1
462	17.9	96	21	3.16	8.25	2.58	11:1
428	18.4	200	45	4.57	16.25	3.56	60:1
436	18.5	207	47	4.65	16.50	3.53	57:1
116	18.5	172	39	4.24	13.75	3.24	33:1
113	18.9	77	18	2.85	5.75	2.02	5:1
464	19.0	166	39	4.18	12.25	2.93	20:1
467	19.9	213	53	4.76	13.50	2.84	17:1
117	19.9	97	24	3.21	6.25	1.95	5:1
465	21.6	80	22	2.95	3.50	1.19	1.4:1
426	22.8	328	97	6.02	9.25	1.53	2:1
446	22.8	305	90	5.80	8.75	1.51	2:1
413	23.2	195	59	4.65	4.50	Under 1	1:1
121	23.3	66	20	2.71	1.50	Under 1	1:1
438	24.4	319	103	6.00	2.50	Under 1	1:1
414	28.1	151	59	4.23	6.50	1.53	2:1
439	33.6	101	51	3.60	13.00	3.89	115:1
460	33.9	39	20	2.24	5.25	2.39	8:1

Interpretation

Some years ago an investigator, upon obtaining such results as these, might have been tempted to interpret them as a violation of the Mendelian mechanism. Today, profoundly impressed with the inviolability of this mechanism, one attempts an interpretation of any novel results in Mendelian terms. Several working hypotheses might fit these data. The one which appealed to the writer was as follows.

The race of corn with which I started must have contained a factor L_1, which had never been identified because it had always existed in the homozygous condition.[1] This factor was linked upon the same chromosome with C, so that the formula of 710 (which was parent of 937–940) was C–L_1, c–L_1. Self-fertilization of 710 would then have yielded three types of grains: 1 colored, C–L_1, C–L_1, which would breed true: 2 colored, C–L_1, c–L_1, which would yield 3:1 on selfing: 1 white, c–L_1, c–L_1. So long as L_1 remained constant, its presence would never be detected. In one of the cells of 710, however, there occurred a mutation which changed the condition C–L_1, c–L_1 to C–L_1, c–l_1. This cell evidently existed early enough in ontogeny to be the ancestor of a group of reproductive cells, although whether it was a group of megaspore or microspore mother cells I cannot tell. In any event, this group of reproductive cells produced gametes of the two types, C–L_1 and c–l_1, the latter type being new. Self-fertilization of 710 caused gametes of this new type to combine with gametes of the two unmodified types, resulting in two new types of zygotes, C–L_1, c–l_1 and c–L_1, c–l_1. Three of the colored grains used (937, 938, 939) contained zygotes of the new type C–L_1, c–l_1; while others (for example, 940) contained zygotes which had been produced by unmutated gametes, and had the formula C–L_1, c–L_1.

It is in self-fertilization of 937, 938, and 939 that the nature of l_1 first becomes apparent. The exact properties of this factor are unknown, but it has the general property of conditioning life, such that any zygote that contains L_1 will be normal, while zygotes (and endosperms) lacking L_1 cannot develop. Gametes lacking L_1, however, may function (that is, this is a zygotic lethal, not a gametic lethal). If C and L_1 were so perfectly linked that no crossing over ever took place between them, plants 937, 938, and 939 would produce, in equal numbers, the two types of gametes, C–L_1 and C–l_1. The results of self-fertilization would then be, 1–C–L_1, C–L_1, normal colored: 2 C–L_1, c–l_1, normal colored: 1 c–l_1, c–l_1, lethal white. Since this last class would not develop, and since the endo-

[1] It seems consistent with the notation employed in *Drosophila* to assign the symbol l_1 to the present factor. The subscript distinguishes it from l, which has been assigned to the yellow seedling character in maize. The symbol can be changed, of course, if the present factor proves identical with any previously found, or if the use of such a symbol violates any convention previously established.

sperm (c–l_1, c–l_1, c–l_1) surrounding it could not develop, mature ears would show colored grains only, and the underlying mechanism would not be detected.

Actually, however, C and L_1 are sufficiently separated upon the chromosome so that considerable crossing over takes place between them. Hence there are produced gametes of the following four types:

Non-crossovers, C–L_1, and c-l_1
Crossovers, C–l_1, and c-L_1

Both male and female gametes of these four types are produced, since in corn crossing over takes place in both microsporogenesis and megasporogenesis (EMERSON 3). Self-fertilization then results in numerous combinations, the significant features of which are as follows:

(1) There are three combinations from which C is absent, so that the resulting grains will be white if they are able to develop at all.

(a) c–l_1, c–l_1 (endosperm c–l_1, c–l_1, c–l_1) is lethal; grains will not develop. This results from combination of gametes c–l_1 and c–l_1 of the non-crossover type. Non-crossover gametes are decidedly more numerous than crossover gametes, hence this combination is relatively frequent.

(b) c–L_1, c–l_1 (endosperm c–L_1, c–L_1, c–l_1 or c–L, c–l_1, c–l_1) is viable; white grains will develop. This results from combination of crossover gamete c–L_1 with non-crossover gamete c–l_1, and is less frequent than (a), but more frequent than (c).

(c) c–L_1, c–L_1 (endosperm c–L_1, c–L_1, c–L_1) is viable; white grains will develop. This results from combination of gametes c–L_1 and c–L_1 of the crossover type, and is relatively rare.

Since (a) is decidedly more frequent than (b) or (c), the majority of potential white grains never develop.

(2) There are several combinations containing C, so that the resulting grains will be colored if they are able to develop at all.

(a) C–l_1, C–l_1 (endosperm C–l, C–l_1, C–l_1) is lethal; grains will not develop. This results from combination of gametes C–l_1 and C–l_1 of the crossover type and is relatively rare.

(b) C–l_1, c–l_1 (endosperm C–l_1, C–l_1, c–l_1, or C–l_1, c–l_1, c–l_1) is lethal; grains will not develop. This results from combination of crossover gamete C–l_1 with non-crossover gamete c–l_1 and is more frequent than (a).

All other combinations containing C will also contain L_1, so that colored grains are developed. The total frequency of these other combinations is so much greater than (a) and (b) that the majority of potential colored grains will develop.

Since the majority of potential white grains never develop and the majority of potential colored grains do develop, the normal Mendelian ratio of 3 colored : 1 white is distorted in the direction of a deficiency of white grains. The amount of this distortion depends upon the relative frequency of crossover and non-crossover gametes. A crossover frequency of about 18 per cent[1] would yield results such as appeared on ears 937, 938, and 939.

Tests of the working hypothesis

The adequacy of this working hypothesis had to be determined by additional tests. These had to be of such a nature that the results predicted according to this working hypothesis would differ sharply from the results otherwise predicted. Three such tests have been applied.

I. As a matter of convenience, the descriptive title "low-white" may be applied to this mutated race which is characterized by producing a deficiency of white grains when self-fertilized. Will this low-white bring a corresponding deficiency in crosses?

The progeny of 938 and 939 are of various types, some giving all colored when self-fertilized, others giving the normal 3:1, and others giving the characteristic deficiency of whites. It is, of course, only the individuals of this last type that in any event would be expected to show any deficiency of whites in crosses. For purposes of demonstration, therefore, one is obliged to use in the cross the same individual which is self-fertilized; since it is only by self-fertilization that one can discover which individuals are the ones to show the deficiency of whites. This has proved difficult, since the supply of pollen is frequently too small for two successful polli-

[1] The percentage of crossing over may be computed by means of the following home-made formula, which gives 17.85 per cent in the present case (n is total grains, and w is white grains):

$$\text{Percentage crossing over} = 100 \cdot \frac{\sqrt{\frac{4n}{3}} - 2\sqrt{\frac{n}{3} - w}}{\sqrt{\frac{4n}{3}}}$$

nations, and it is usually impossible to develop two good ears upon the same plant. As a consequence, although many crosses have been performed with the low-white race, it is in only a few cases that I have been successful in simultaneously selfing and crossing the same individual. These few cases appear in table V. In each case the individual of the low-white race which was used in the cross was known to show a decided deficiency of whites upon self-fertilization.

The cross in question is with "C-tester," a race of which the formula is known to be c–L_1, c–L_1. The normal result of crossing this with plants heterozygous for C is a ratio of 1 colored: 1 colorless. Do the low-whites used in this cross bring a deficiency of whites in the result?

TABLE V

RESULTS OF CROSSING LOW-WHITE WITH C-TESTER

Ear	Low-white as which parent	Percentage white	Colored	Colorless	P.E.	Deviation	Dev./P.E.	Probability
21.130.....	Male	53.0	195	222	6.89	13.5	1.97	4.4:1
531.....	Female	56.0	51	65	3.63	7.0	1.93	4.2:1
183.....	Female	51.0	103	107	4.89	2.0	Under 1	1:1
24.40.....	Male	48.7	182	173	6.35	4.5	Under 1	1:1
481.....	Female	48.4	162	152	5.99	5.0	Under 1	1:1
24.13.....	Male	43.5	39	30	2.80	4.5	1.61	2.6:1
Totals....	.....	.....	732	749	23.42	8.5	Under 1	1:1

It is evident from table V that no deficiency of whites appears in crosses between low-white and C-tester. Nor would one expect any deficiency from the assumption of a zygotic lethal; for the C-tester must always contribute an L_1, so that the resulting zygote (and endosperm) will be viable.

The exact combinations resulting from this cross are as follows. C-tester produces only one type of gamete, c–L_1. Low-white produces four types: non-crossover gametes C–L_1 and c–l_1 in equal frequency (n), and crossover gametes C–l_1 and c–L_1 in equal frequency (m, which is less than n). Hence the four possible combinations and their frequencies are: (n) C–L_1, c–L_1, colored viable: (n) c–l_1, c–L_1, white viable: (m) C–l_1, c–L_1, colored viable: (m) c–L_1, c–L_1, white viable. Then total colored is (n) plus (m), and total white is also (n) plus (m).

Many more crosses were made in which the exact composition of

the low-white parent used was not identified by self-fertilization. The resulting ratios in no case showed a significant deficiency of whites.

II. One might hardly expect to obtain from the low-white race a daughter race which was "high-white," that is, one which was characterized by producing an excess of whites (deficiency of colored grains) when self-fertilized. Yet this very result must be predicted from the assumption of the zygotic lethal.

Among the numerous combinations which result from self-fertilizing the low-white, there is a rare one with the formula $C\text{–}l_1, c\text{–}L_1$. In this case the previous linkage relationships are reversed, l_1 now

TABLE VI

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
547	0.0	200	0	.....	.....	.....	.....
549	20.2	87	22	3.05	5.25	1.72	3:1
546	21.9	50	14	2.34	2.00	Under 1	1:1
551	25.0	18	6	1.43	0.00	Under 1	1:1
543	25.4	182	62	4.56	1.00	Under 1	1:1
540	27.3	8	3	0.97	0.25	Under 1	1:1
545	30.9	65	29	2.83	5.50	1.94	4:1
542	34.4	82	43	3.27	11.75	3.59	63:1
552	34.6	34	18	2.11	5.00	2.37	8:1
541	36.4	82	47	3.32	14.75	4.44	370:1
544	36.9	41	24	2.35	7.75	3.29	37:1
550	39.3	71	46	3.16	16.75	5.30	5,000:1
548	40.0	30	20	2.07	7.50	3.62	65:1

being linked in the same chromosome with C, and L_1 with c. What result would be expected from self-fertilizing such an individual?

The same forces which brought a deficiency of whites in the previous case must bring a deficiency of colored grains here. Many potential colored grains will be eliminated by combination of the numerous non-crossover gametes $C\text{–}l_1$ and $C\text{–}l_1$, while only a few potential whites will be eliminated by combination of the less numerous crossover gametes $c\text{–}l_1$ and $c\text{–}l_1$. Hence the normal 3:1 ratio should be distorted in the direction of a deficiency of colored grains. Calculations based upon the same values used before call for a ratio of about 67 per cent colored: 33 per cent white.

In tables III and IV appear a few ratios which are suspiciously close to this predicted high-white condition. Are such individuals truly high-whites, or do they represent merely chance deviations? This question is answered in table VI, the results of self-fertilizing

progeny of the colored grains which occurred on one of these suspected high-whites.

It is evident from table VI that the deficiency of colored grains is heritable. As predicted, a high-white race is produced by the low-white parent race.

TABLE VII

RESULTS OF SELFING PLANTS OF 531 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
24.110.....	26.6	149	54	4.16	3.25	Under 1	1:1
24.114.....	26.8	203	77	4.89	7.00	1.43	2:1
24.115.....	30.6	143	63	4.19	11.50	2.74	14:1
24.112.....	30.9	183	82	4.75	15.75	3.32	40:1
24.113.....	34.9	123	66	4.02	18.75	4.64	550:1

TABLE VIII

RESULTS OF SELFING PLANTS OF 183 FAMILY

Ear	Percentage white	Colored	White	P.E.	Deviation	Dev./P.E.	Probability
24.94.....	22.7	341	100	6.13	10.25	1.79	3:1
24.91.....	23.5	287	88	5.66	5.75	1.02	1.03:1
24.83.....	23.8	350	109	6.26	5.75	Under 1	1:1
24.92.....	24.6	362	118	6.40	2.00	Under 1	1:1
24.80.....	25.3	204	69	4.83	1.75	Under 1	1:1
24.88.....	25.4	247	84	5.31	1.25	Under 1	1:1
24.81.....	25.4	288	98	5.74	1.50	Under 1	1:1
24.82.....	25.5	216	74	4.97	1.50	Under 1	1:1
24.84.....	25.6	232	80	5.16	2.00	Under 1	1:1
24.101.....	25.9	281	98	5.69	3.25	Under 1	1:1
24.100.....	25.9	103	36	3.44	1.25	Under 1	1:1
24.99.....	26.2	225	80	5.10	3.75	Under 1	1:1
24.86.....	28.2	102	40	3.48	4.50	1.29	1.6:1
24.95.....	28.8	227	92	5.22	12.25	2.35	8:1
24.85.....	30.5	157	69	4.39	12.50	2.85	17:1
24.96.....	31.0	20	9	1.57	1.75	1.11	1.2:1
24.89.....	32.7	165	80	4.57	18.75	4.10	200:1
24.98.....	32.8	180	88	4.78	21.00	4.38	300:1
24.97.....	34.2	156	81	4.50	21.75	4.83	900:1
24.87.....	34.5	91	48	3.44	13.25	3.85	100:1
24.93.....	35.0	130	70	4.13	20.00	4.84	900:1
24.90.....	36.0	103	58	3.71	17.75	4.79	800:1

III. In the cross between low-white and C-tester, the lethal capacity of the former was concealed, just as any recessive is concealed by its dominant allelomorph. One would expect, therefore, that in the following generation this lethal capacity would become

manifest in at least some of the offspring. Since low-white is the condition that went into the cross, should not low-white be the condition that segregates out again in the second generation? Should not some of the progeny of low-white × C-tester reveal a deficiency of white grains when self-fertilized?

As a matter of fact, this is not what would be predicted from the assumption of the zygotic lethal. The colored grains produced by crossing low-white with C-tester should be of two types, C–L_1, c–L_1 and C–l_1, c–L_1, the former being the more frequent. Self-fertilizing the former should give the normal 3:1 ratio, while self-fertilizing the latter should give a deficiency of *colored* grains, since C–l_1, c–L_1 is the formula characteristic of the high-white race. Colored grains of ears 531 and 183 (table V) were planted, and the resulting plants self-fertilized. The results appear in tables VII and VIII.

Again the working hypothesis is confirmed, for it is high-white that regularly segregates out of the cross between low-white and C-tester.

Secondary lines of evidence

In addition to the preceding tests, there are two other lines of evidence that should be mentioned. One lies in the ratio between those colored grains which breed true and those which do not; that is, the ratio between plants homozygous for C and those heterozygous. In the original race, of course, self-fertilization of C–L_1, c–L_1 gave 1 C–L_1, C–L_1 : 2 C–L_1, c–L_1 : 1 c–L_1, c–L_1. Consequently, when colored grains were planted and the resulting plants selfed, one-third of these were expected to give all colored, while the other two-thirds gave some (25 per cent) white grains upon the ears. Previous work has adequately verified this prediction, which is illustrated, upon a small scale, by the progeny of 940 (table II).

The question now arises whether this ratio between pure and heterozygous colored will be different in the low-white and high-white races. Using the first set of data that were available, the ratios which appeared on ears 937–939, one finds a total ratio of 608 colored : 74 white, or 10.85 per cent white, which corresponds to a crossover frequency between C and L_1 of approximately 18 per cent. Computations based on this value call for the following relative

frequencies among the different genotypes of the colored class in the low-white race:

Giving all colored when selfed.....36.2 per cent
Giving some white when selfed.....63.7 per cent

How well these expectations are satisfied by the results obtained (from selfing colored class of 938 and 939) can be judged from table IX.

TABLE IX

RELATIVE FREQUENCY OF PLANTS HOMOZYGOUS AND HETEROZYGOUS FOR C IN COLORED CLASS OF LOW-WHITE RACE

	Giving all colored when selfed	Giving some white when selfed
Expected in normal race	25.66	51.33
Observed in low-white	30.00	47.00
Expected in low-white	28.00	49.00

Computations based upon the same crossover value call for the following relative frequencies among the different genotypes of colored grains in the high-white race:

Giving all colored when selfed.....16.1 per cent
Giving some white when selfed.....83.9 per cent

These expectations are compared with the results obtained in table X.

TABLE X

RELATIVE FREQUENCY OF PLANTS HOMOZYGOUS AND HETEROZYGOUS FOR C IN COLORED CLASS OF HIGH-WHITE RACE

	Giving all colored when selfed	Giving some white when selfed
Expected in normal race	4.33	8.66
Observed in low-white	1.00	12.00
Expected in low-white	2.00	11.00

The data in these last tables are meager; yet they at least tend to confirm rather than refute the assumption of a zygotic lethal.

Another secondary line of evidence that might be considered lies in the physical condition of the ears themselves. Those ears

which show either an excess or a deficiency of white grains have presumably had 25 per cent of their potential grains eliminated by the action of the lethal. Hence one might expect such ears to bear approximately three-quarters as many total grains as do the ears with the normal ratio of 3 colored : 1 white, where there is no lethal. Whether this is actually true cannot be determined satisfactorily from examination of the ears produced by self-fertilization of low-white and high-white. When corn has been inbred for several generations, the tendency for "weaklings" to segregate out results in a population of the greatest diversity with respect to the number of grains per ear. The enormous variation due to these causes would

TABLE XI

TOTAL GRAINS PRODUCED ON EARS OF POPULATION RECORDED IN TABLE VIII

Ear	Percentage white	Total grains	Ear	Percentage white	Total grains
24.94.....	22.7	127.4	24.99.....	26.2	88.3
24.91.....	23.5	108.5	24.86.....	28.2	41.1
24.83.....	23.8	132.0	24.95.....	28.8	92.3
24.92.....	24.6	138.9	24.85.....	30.5	65.4
24.80.....	25.3	70.0	24.89.....	32.7	70.9
24.88.....	25.4	95.8	24.98.....	32.8	77.6
24.81.....	25.4	111.7	24.97.....	34.2	68.6
24.82.....	25.5	83.9	24.87.....	34.5	40.2
24.84.....	25.6	90.3	24.93.....	35.0	57.9
24.101.....	25.9	109.7	24.90.....	36.0	46.6

obscure hopelessly the effect of the lethal upon total number of grains.

There is available, however, another type of population which does not have these shortcomings. The cross between low-white and C-tester brought with it the benefits of heterosis. When the resulting (colored) grains were planted, there was produced a population of relatively vigorous plants with relatively uniform ears. Some of these ears were high-white, due to the activity of the lethal; others lacked the lethal and showed normal 3:1 ratios (table VIII). Here if anywhere the effect of the lethal in reducing the total number of grains should be noticeable. The average number of grains produced on ears which showed the 3:1 ratio was 345.5. In table XI is

recorded the relative number of grains produced on all ears of this population, 100 per cent being equivalent to 345.5 grains.

Clearly there is a tendency for the high-whites to carry less total grains than do the normals. If this is not due to the activity of the lethal, what can be the explanation?

Bearing upon genetics of maize

Two points of interest appear in the bearing of these results upon genetical research in maize. First, as to the nature and identity of the factor $L_1 l_1$. Several lethal factors of the chlorophyll-deficiency type are already well known in maize. In these cases the lethal effect appears in the seedling stage, after the food supply in the seed has been exhausted. For something comparable with the present case, however, one must look to examples of a defect or lethal effect appearing still earlier in ontogeny. There have been reported seeds with no embryo, although the endosperm appears normal (**1**); also seeds in which both embryo and endosperm, although present, are clearly defective, giving a semi-lethal effect (**6**). Finally, JONES (**5**) reports a character of which the extreme expression is absence of both embryo and endosperm, although the pericarp is present. This last situation, which has been shown to be due to a Mendelian recessive, and which, of course, gives a lethal result, certainly represents the same type of behavior as the case in hand. Whether the two cases are identical, may be determined in the future. Some of the ears occurring in these experiments appear to be carrying a few minute "empty pericarps." These are so tiny and irregular, however, that as yet it has not been possible to ascertain any systematic relationship between such aborted seeds and the lethal.

The other point of interest arises from the linkage group into which the present factor falls. Already HUTCHINSON (**4**) has established the linkage relationships of four endosperm factors, I–C–Sh–Wx. Presumably the present factor will fall into this same group, and should prove useful in enlarging knowledge of linkage phenomena in maize.

Frequency of crossing-over

The tentative crossover frequency of 18 per cent was computed from the ratios which appeared on ears 937, 938, and 939. If this

frequency were constant, the low-whites appearing among the progeny of 938 and 939 should all have possessed close to 10.85 per cent white grains. Instead there appeared an unexpected amount of variation in the relative number of white grains. It was felt that this variation was too wide to be due merely to chance.

Of the various possible explanations of this situation, the one that at present seems to be the most likely is that there is an underlying variation in the frequency of crossing-over. This tentative assumption carries with it the question as to whether environmental or genetic factors are responsible for modifying the crossover frequency; both have been demonstrated in the fruit-fly (**2, 8, 9**).

With this in mind, selection experiments are now being carried on with the progeny of 938 and 939, and already data have been obtained to justify the following report. Low low-white (6.8 per cent) produces offspring characterized by a lower percentage of white grains than the average (for low-white), and a relatively higher proportion of low-whites as compared with normal 3:1 ratios. Medium (12.5 per cent) and high (18.5 per cent) low-whites produce offspring characterized respectively by a medium and high percentage of white grains, and by a medium and low proportion of low-whites as compared with normal 3:1 ratios. In short, the variation in the relative number of white grains in the low-white race is of genetic significance.

Discussion

The explanation of distorted Mendelian ratios by means of an imaginary zygotic lethal may appear fantastic, yet I believe it is quite consistent with recent discoveries. It is now well established that true mutations are simple factor changes, and that these changes are of a random nature (so far as we can as yet determine). Is it not to be expected that random changes, occurring in the complicated mechanism of the living organism, will more often impair the efficiency than improve it? Certainly this is consistent with the facts, for mutants are more often degenerates than improvements. In fact, the investigation of recent years conveys the impression that lethal mutations are the commonest of all; MULLER and ALTENBURG (**7**) provide what may be regarded as a demonstration of this point in the fruit fly.

That most mutations are lethal would be more evident if lethal mutations were not so frequently overlooked. Dominant lethal mutations cannot be identified with certainty. Recessive lethal mutations are identified only when (as in the present case) they are linked with some previously known factor that is under observation.

Although dominant and recessive mutations may in reality be equally common, the latter appear to be much more common than the former. This is merely the result of the two facts, that most mutations are lethal, and that recessive lethal mutations can be identified, while dominant lethal mutations cannot. Statistics on observed mutations, therefore, should not be used to demonstrate that there is a greater tendency for recessive than dominant mutations.

The adequacy of mutation as a basis for progressive evolution depends upon whether mutants are ever improvements. Faith in the evolutionary value of mutation has been shaken by claims that no mutant has ever been shown to be an improvement. In answer, two points should be suggested. (1) Would it not be very difficult to recognize as such those rare improvements, or potential improvements, which nature provides through mutation? (2) It is known that dominants may be added by mutation; and this is the only known mode of origin. Many existing dominants are clearly improvements over the corresponding recessives. Have not such dominants been added by mutation in the past?

In objection to this last proposition, the following might be urged. Granting that many existing dominants are such decided improvements over the corresponding recessives that the dominant condition is viable and the recessive lethal, how then may one imagine the circumstances of their origin? For example, can one believe that L_I (of the present experiments) was suddenly produced by mutation in an organism $l_I\ l_I$, when such an organism could not have lived? There can be only one answer, $l_I\ l_I$ must have lived. The organism of today is more complicated than the organism of the past; its mode of life involves dependence upon a greater number of mechanical adjustments. Once there existed the organism $l_I\ l_I$. Then L_I appeared and was subsequently utilized in connection with a new type of adaptation. The final perfection of this adaptation

was a step in evolutionary progress; but the price paid was absolute dependence upon L_1. In such a case evolution is irreversible.

In short, if one attempts to visualize evolution in terms of single factor changes alone, the origin of our most important factors by mutation appears logically unsound. The important steps in evolutionary progress doubtless involve several factor changes. One can live on the ground floor without walls or a roof; but if walls and a roof appear, and the family takes up its abode on the roof, the sudden removal of either walls or roof may produce a lethal effect. Our modern organisms are skyscrapers. One should not attempt, therefore, to visualize evolution in terms of single factor changes alone, any more than one should believe that Mendelian factors are the only forces operating to determine expressed hereditary characters.

Summary

1. A race of corn was discovered in which self-fertilization of genotype Cc produces, not 25 per cent white grains, but 10.85 per cent; that is, the normal 3:1 ratio has been distorted in the direction of a deficiency of the recessive class.

2. To account for these results, a working hypothesis is proposed which involves a zygotic lethal factor partially linked with c. If the original genotype C–L_1, c–L_1 mutated to C–L_1, c–l_1, self-fertilization would eliminate many of the potential whites through the action of the lethal. The exact ratio resulting would depend upon the frequency of crossing-over between c and l_1; the observed results correspond to about 18 per cent crossing-over.

3. This hypothesis has been confirmed by three breeding tests. (a) Individuals of genotype C–L_1, c–l_1, which give a deficiency of whites upon self-fertilization, give no deficiency in crosses with C–tester (c–l_1, c–L_1). (b) The low-white race has thrown high-white races (33 per cent white) of composition C–l_1, c–L_1. (c) Part of the colored grains from low-white × C-tester behave as high-white upon self-fertilization.

4. Subsidiary lines of evidence are (a) modified proportion between homozygous and heterozygous colored grains, and (b) deficiency in total number of grains upon those self-fertilized ears which carry the lethal.

5. This factor is very similar to, and possibly identical with, one previously reported by JONES. Presumably it falls into the linkage group I–C–Sh–Wx, which has been worked out by HUTCHINSON.

6. There are indications that frequency of crossing-over may be modified by genetic factors. Data upon this will be presented in a later paper.

7. It is emphasized that lethals are the commonest of mutations. Ideas are presented which may assist in visualizing progressive evolution by factor changes.

I am greatly indebted to ANDREW STAUFFER and R. O. EARL, who rendered valuable assistance in the field.

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SPATHYEMA FOETIDA

J. MARION SHULL

(WITH PLATES I–IV)

The history of the "skunk cabbage" dates back to its earliest mention by JOHN JOSSELYN, in *New England's rarities discovered*, published in 1672, where he includes it among such plants as are "proper to the country, and have no name." A crudely engraved illustration enables us today to recognize the subject of his description, but if there were any question as to its identity, his statement that "the whole plant sents as strong as a fox" would tend to remove any lingering doubt. Ever since those early days the skunk cabbage has been an object of interest both to botanist and layman; to the latter mainly perhaps because of its pungent, skunklike odor, and because of its seeming extreme precocity in the matter of bloom. In many regions it has come to be looked upon as the earliest of spring flowers. Not having been seen earlier in the season, and then being found in an already spent condition has led to a popular belief that these inflorescences are of extremely rapid, almost spontaneous growth. They are compared with mushrooms for rapidity of development, but this is a mistake.

Impressed by the popular belief in this rapidity of growth, some years ago the writer began a series of observations on a station near Cleveland Park in the District of Columbia, only to find that in this latitude *Spathyema foetida* was hardly to be classed among spring blooming flowers at all. In this locality flowers might have been found in full anthesis at any time between the latter part of November, 1918, and early March of the following spring, a winter of no extreme or long continued cold. Probably few seasons pass at this station without some of the more precocious spathes reaching maturity during the fall or during somewhat protracted periods of warmth in winter. Very likely in more northern regions this fall and winter blooming would occur with less frequency, but it has occasionally been reported from places farther north, and in some cases has led to the mistaken assumption

that it may sometimes flower more than once in the same year. This is not the case, however, since the plant that blooms itself out in November or December does not bloom again the following spring, there being but one annual blooming period for each individual plant, whether it come in fall, winter, or spring. In the region under consideration fall blooming may be looked upon as habitual. It is noteworthy, however, that while these early spathes are developing, all of which are usually destined to be frozen later, others under apparently identical environment as to heat, light, and moisture make no considerable response to the unseasonable warmth, but remain almost entirely quiescent. Thus it would appear that the species is well fortified against such possible disaster as might result from freezing if all the plants pushed their spathes forward with equal promptness under the stimulus of unwonted warmth.

The growth of these earliest appearing spathes, moreover, is not by leaps and bounds, as is the case with so many other spring flowers, but is a gradual and fairly continual process, mostly at relatively low temperatures. Spathes carefully measured and marked with waterpoof India ink were observed at intervals from November 6 to December 31, 1918. Later observations were discontinued at this point, owing to accidental damage to a number of the marked plants, but up to this time each successive examination had showed greater or less increase over the next preceding record, the spathes during the whole period having made an average increase of about 4 mm. in diameter and 7 mm. in height, notwithstanding that during much of the time the ground was frozen at the surface. It is probably true that the plant maintains an internal temperature somewhat higher than that of the surrounding air and soil in winter, so that it is not uncommon to find specimens with a small circle about them freed of snow or ice somewhat in advance of its disappearance elsewhere, and under circumstances that apparently cannot be explained except on the assumption of internal warmth. While not being able to find any published data to bear out the statement, nor having had opportunity to corroborate it in any way, it is the writer's understanding that actual measurement of this internal warmth was made some years ago

which showed an internal temperature several degrees higher than that of the surrounding environment. If this is correct it would partially account for the gradual if slow development throughout the greater part of the winter season, as indicated by the measurements here reported.

That the finding of flowers in early autumn led to reports of more than one flowering season per year has already been noted, and can readily be understood, but it is rather difficult to understand how ENGLER (2) should have made the mistake of thinking that *Spathyema* bloomed only in alternate years, foliage one year and flower the next. It is true that to the layman the plant might be rather puzzling, for in the fall or in early spring he might see the spathes with practically no indication of any foliage growth (fig. 12). If in spring, a few weeks later there would be a great mass of light green leafage (fig. 13), which to the casual observer would have no obvious connection with the curious flower found upon the former visit. A few months later he might find an unusual looking object about the size of an average potato, roughened on the outside in a way somewhat suggestive of a pineapple (fig. 15), but so entirely detached from any plant growth then visible as to be beyond his powers of identification, so completely does the foliage disappear. Still later he might find a somewhat scattered handful of something suggestive of ground nuts in appearance, the seeds, now divested of the soft, spongy tissue of the fruit (fig. 14), without even associating them with the peculiar object previously found.

Many features of this interesting plant are not well known, even among botanists. ENGLER pointed out that most of the Araceae are sympodial in their manner of growth, and that *Spathyema* was no exception in this respect, but as he depended on a single dried herbarium specimen for his detailed account of it, it is hardly surprising that he should have gone astray in his description of the succession shoot (1), where he indicates not only too many sheaths or reduced leaves together, but too great a number of ordinary leaves occurring between succeeding spathes. Morphologically, of course, we should make no distinction between these wintering-over sheaths and the more fully expanded leaves of the active vegetative period. Both vary somewhat in number in

different plants, or may grade one into the other to some extent; but so far as the succession shoot is concerned, it invariably consists of two of these units, following which the terminal bud is consumed, one might say, in the development of the inflorescence. Of the two buds in the axils of these two leaves, the upper must always be developed into the new terminal shoot to take the place of the former terminal now converted into a spathe and spadix. The lower of the pair of buds remains of microscopic size until such time as it approaches the outer region of the crown, when it may distinctly be seen without a lens. These may be looked upon as reserve buds and do not ordinarily develop, apparently never do so while the original crown continues to function, and perhaps this is what ROSENDAHL (6) really means when he says, "in no case . . . . do they develop into lateral shoots." That these lateral buds do have the power to develop, presumably after many years of dormancy, and do in consequence provide insurance against the destruction of the crown, is well shown in fig. 18, where a portion of an old trunk has freely given rise to lateral growths. Doubtless these lateral growths, had they remained undisturbed, would presently by their very manner of growth have broken themselves free from the parent trunk, and in a few years would have presented all the appearance of individual plants developed from the seed.

In studying the morphology of this plant, FOERSTE (3) explains at some length the deceptiveness of appearances met with when dissecting a mature crown. At first sight it would seem, from their location near the outer circle, that the spathes should have been developed from axillary buds, but on closer examination the ordinary relations of such buds do not hold. There is the added difficulty, of course, that each leaf base makes a practically complete circle, with a series of exactly similar fibrovascular bundles, or shall we say leaf traces, no one of which can be positively determined upon as representing the midvein, so that it is almost impossible to determine the true axis of the leaf. Near the outer portion the bud occurring in the axils of alternate leaves helps to locate the exact axil of the lower leaf of each shoot, but these buds soon become invisible as dissection progresses, and even under a high power lens are frequently missed.

Once it is realized, however, that each spathe in its turn represents the terminal of the whole plant at a certain period, each yielding in its turn to the bud next below with clocklike precision as long as the plant may live, it is easier to interpret what is actually happening. An attempt to diagram this condition is shown in fig. 1, where each circle represents a complete succession shoot with its two leaves, but for clearness the individual leaves are not indicated as extending entirely around the circle. A spathe stands outside the outer circle, the latter representing the axillary bud lying next below the spathe; that is, in the axil of a leaf of the preceding year, which has disappeared. The first or lower leaf is shown with a small white circle at the center, to indicate the presence and location of the lower lateral bud, destined to remain dormant until needed by reason of accident to the upper portion of the plant. The second leaf lies approximately two-fifths around the circle, and in its axil lies the bud represented by the second circle from the outside. This circle lies directly between the second or upper leaf and what is for the moment the axis of the plant here represented by the second spathe. Thus it is clear that the developing and great expansion of this upper of the pair of lateral buds will push the axis or spathe off to a considerable distance, but always directly opposite the upper leaf, the spathe appearing clasped within the margins of the leaf base as seen in figs. 8 and 10. In fig. 1 each succeeding circle, proceeding toward the center, represents the bud lying in the axil of the second leaf of the next preceding circle or shoot, but no attempt has been made to show the large number of such succession shoots that are actually present in any strong crown.

Before dealing further with minute details of dissection, however, it may be well to trace somewhat more connectedly the development of the plant from seed. First, the seed itself is quite unlike most seeds in that there are no seed coats in evidence, these having disappeared during development of the ovary through the absorption of the endosperm and integuments, as reported by ROSENDAHL (5). The mature seeds average about 1 cm. in greatest diameter, and are nearly spherical, except when distorted by pressure due to crowding. Externally they are light brown, while the dense firm

flesh, homogeneous throughout, is of a peculiar bluish white. Opposite the hilum lies the plumule, imbedded in the starchy tuber-like mass of the seed itself, a small slit on the surface adjoining the micropyle serving to show its location. A median longitudinal section of the seed is shown in fig. 6, where the vascular relationship may be noted. The plumule shown under greater magnification (fig. 5) and again in cross-section (fig. 4) is in no respect different from the lateral bud shown in fig. 3, all the same magnification, and quite a number of rudimentary leaves are already clearly in evidence. NUTTALL (4) describes the seed as not appearing to "possess anything like a proper cotyledon," declaring that "in place of a cotyledon there is a sheathing stipule similar to that which is ever after produced; in fact it is viviparous." He is mistaken, of course, in attributing this sheath to stipular origin, whether in the plumule or in the mature plant, since in either case they are merely reduced leaves. ROSENDAHL (5), on the other hand, looks upon the entire fleshy body of the seed as the "cotyledon," in which the plumule is all but completely buried. Shall we say that this seed has already germinated, and so agree with NUTTALL in describing the plant as viviparous? This must depend of course on our definition of germination. In any case this would seem to be the condition in which the seed is prepared to undergo whatever period of inactivity it is capable of sustaining. So far as the writer knows, no determinations have ever been made as to whether or to what extent the seed may undergo drying and still retain its power to develop, or how long it may remain inactive yet viable with a minimum requirement of moisture to prevent shriveling.

The young plant shown still attached to the seed in fig. 2 is thought to be a one-year old plant. At this stage of development, and apparently for a number of years to follow, the growth is monopodial and relatively slow. No one seems to have determined how long the monopodial stage continues, but it is evident that some of these young plants, making often only several leaves in a season, must continue many years before they outgrow this period of infancy. During this time the life of *Spathyema* constitutes one monotonous unfolding of leaf after leaf in exactly the same manner in slow succession, broken only by such periods of rest as the

changing seasons impose, each leaf rolled round and round on itself and completely inclosing the next succeeding leaf, and all rolled in the same direction on the same plant. Only in a single instance among the number of young plants examined was a change in the direction of rolling of the leaf found, and then it manifested itself in serious deformity of the plant. Probably there is considerable variation in the time required to outgrow the monopodial stage, but the crown must have attained a diameter of 20–25 mm. before the change takes place. This is an important period in the life of the young plant, for at this point the younger stage disappears with the converting of the terminal bud for the first time into an inflorescence. After this it will with absolute precision produce two leaves and then an inflorescence, unless through some mishap the growing point should be destroyed, a contingency not often to be reckoned with, since this same point is usually maintained several inches below the surface of the ground and well beyond the reach of animals that sometimes feed upon the leafage; and while damage might be caused by trampling of heavy animals, the mere fact of its swamp habitat would insure against this happening with any frequency.

A young plant having cut off its terminal bud for the first time to form an inflorescence would still be flowerless for a considerable period, possibly for a couple of years; but it will be better at this point to start with a large crown and follow step by step the dissection in detail. The results are shown in condensed form in table I. A large number of crowns of varying sizes were dissected and recorded, and the figures here given are the direct record of a very strong plant with a crown 41 mm. in diameter and as found in November 1918. First there is a large wintering over sheath without leaf expansion, rolled on itself as indicated in the table when looking down on the plant. In the clasp of the leaf base margins there is a wasted spathe. In most plants of this size this spathe would have been sound and there would be no remnants of last year's inflorescence in evidence. In other words, most strong plants at this station produce two mature spathes each season, but there are weaker plants with only one, and yet others that produce three, while an extreme of four has been reported. Next there is

another sheath with slight expansion of leaf at the apex, but the edges overlap in the opposite direction and there is no spathe present. The third sheath, again with slight leaf expansion and

TABLE I

RELATIONSHIPS IN LARGE CROWN OF *Spathyema*, 41 MM. DIAMETER, DISSECTED IN NOVEMBER

No.	Condition	Direction of roll	Spathe	Season
1	Large sheath, no leaf expansion	↻	+aborted	
2	" " slight expansion	↺	o	
3	" " slight expansion	↻	+live 28×92 mm.	Spring
4	" " considerable expansion	↺	o	1919
5	Leaf fully formed	↻	+aborted	
6	" " "	↺	o	
7	" " "	↻	+aborted	
8	" " "	↺	o	
9	" " "	↻	+aborted	
10	" " "	↺	o	
11	" " "	↻	+aborted	
12	" " "	↺	o	
13	" " "	↻	+live 4×10 mm.	Spring
14	Rudimentary leaf	↺	o	1920
15	" "	↻	+live 3×6 mm.	
16	" "	↺	o	
17	" "	↻	+live 2×4 mm.	
18	" "	↺	o	
19	" "	↻	+live	
20	" "	↺	o	
21	" "	.....	+live	
22	" "	.....	o	
23	" "	.....	+live	
24	" "	.....	o	
25	" "	.....	+live	Spring
26	" "	.....	o	1921
27	" "	.....	+live	
28	" "	.....	o	
29	" "	.....	+live	
30	" "	.....	o	
31	" "	.....	+live	

again reversing the direction of its rolling, clasps a live spathe 28 mm. wide and 92 mm. in height, not yet in anthesis but practically fully grown, and apparently destined to bloom in the spring of 1919. This is followed by a fourth sheath which now shows considerable leaf expansion and is again minus the spathe. Very careful inspection at its axil would probably reveal a minute vegeta-

tive bud, but even at this early point in the dissection they begin to be difficult to find. Fifth in order is found a fully formed leaf, lacking only expansion to make the very large leaf common to the plant as seen in early summer. Beginning at one edge it is tightly rolled many times around itself with much crumpling. Entirely within it lies the next leaf, and within the clasp of the basal margins of the petiole is found a spathe that is no longer alive, as is shown by the discoloration and the membranaceous character of the tissues. The succeeding leaves are much the same, except that they grow smaller and smaller, the sixth, eighth, tenth, and twelfth without accompanying spathes, the seventh, ninth, and eleventh clasping aborted spathes. The thirteenth leaf (fig. 8), however, while still a fully formed leaf like its predecessors, clasps at its base a live spathe 4 mm. wide and 10 mm. high, obviously destined to come into flower one year later than the large spathe associated with the third leaf, or sheath, already mentioned, or in the spring of 1920. Now there is found an abrupt change in character from 13 to 14, the latter (fig. 9) being very definitely rudimentary and destined more than a year in advance to become the wintering-over sheath to protect the bud through that period of cold. Figs. 8 and 9 show both leaves to the same scale. From this point on all the spathes are alive, despite the fact that only 13, 15, and 17, in that immediate region, have the slightest possibility of coming to maturity, and probably not more than two of these will escape. Up to the twentieth leaf dissected off, it has still been possible to determine which way the leaf rolls, and, as shown in the table, there is no break in the rule of constant reversal of direction. The seventeenth leaf with its clasped spathe is shown in fig. 10, and beyond this the height of the leaf is reduced more and more. They become mammiform, occupying a pronounced depression at the center of the crown, whence they were dissected to the final number of 31 (fig. 11), the spathe accompanying this last leaf being shown in fig. 7, and photomicrographs of another at about the same stage in figs. 16 and 17. Since the scheme of annual development is so clearly indicated, just as the number of leaves in the bud of many oaks and other trees is definitely fixed long before growth begins in the spring, it is allowable to set aside a

second series of equal number, and assume that this will represent the following year's quota of leaves. Thus it will appear from table I, that 25 and 27 of this crown, had they been permitted to grow, would have provided the bloom in the spring of 1921, some twenty-seven months after the date of this dissection. The remaining spathes there indicated would fall into the third series of aborted spathes, abortion due to occur two years later.

One is tempted to speculate concerning this suppression of more than fifty per cent of the spathes. This behavior is noted by ROSENDAHL (**6**), who attempts to explain it on the basis of habit, a habit acquired during an earlier assumed tropical existence, and which it has been unable to leave behind when forced to live under the adverse conditions of alternating winter and summer. In the opinion of the writer this explanation is untenable. In any case such an assumption seems quite unnecessary. The plant is only functioning in a way that is common throughout the plant world. The very manner of its growth, that of converting its terminal bud into an inflorescence at every second turn, leaves no possibility of reducing their number. Consider that the individual fruit is of large size (figs. 14, 15), containing many large seeds consisting of dense albumen. Also, notwithstanding the supposed tropical origin of the species, the plant confines its vegetative growth to a brief two months of the spring, after which the foliage dies off. Obviously it would be impossible for each pair of leaves, large as they are, to provide sufficient photosynthetic action to meet all the needs of the plant otherwise, and also mature a fruit of such size and character. An apple tree, even with the most perfect pollination, drops most of its fruit in order that it may properly mature the remainder. So with *Spathyema*, it is necessary to secure a proper balance between photosynthesis and seed production, which is brought about by the abortion of approximately two-thirds of the spathes inescapably laid down. As to just how this abortion of these particular spathes, attributed by ROSENDAHL (**6**) to the rigors of winter, is brought about, whether by pressure, by diversion of growth materials, or otherwise, would be a problem for the physiologist. The writer is inclined to think, however, that growth pressure is the means of accomplishing the desirable reduction in number.

To revert to the matter of leaf disposition in the bud and the change that takes place on the transition from monopodial to sympodial manner of growth, it would be interesting to know whether or not the constant alternation of direction is a natural consequence of the latter manner, in a succession shoot of but two leaves. At any rate it is plainly a great advantage in a large, compact, rapidly growing bud, to secure easy and unrestricted expansion, free from all friction of adjacent unrolling leaf surfaces. This may be well illustrated by taking half a dozen strips of paper, rolling the first very closely on itself and the second outside of this but in the opposite direction, and so continuing until all are rolled together. On releasing them note how they all expand at once and throughout the entire roll without the slightest obstruction. This is just what happens with *Spathyema* buds during the extremely rapid vegetative growth of early spring.

Apparently all who have undertaken a detailed account of this species have hesitated over the question of its phyllotaxy, indicating that the leaf arrangement of the mature plants was of a rather high ratio, ROSENDAHL (**6**) stating it positively as 5–13, but all seem agreed that in the young plant the leaf arrangement is of a lower order, although not risking to say with any assurance just what. ENGLER thought it probably 1–2 in the young plant, changing later to a higher ratio. FOERSTE, while noting the approximate 1–2 appearance of the first pair of leaves, and mentioning the probability of a more complicated arrangement, finally decides that it "seemed reasonable to consider this a case of 1–3 phyllotaxy," crediting such divergence as was more or less apparent to displacements necessarily taking place in so complicated and crowded a structure. There is no denying the difficulty involved in trying to determine the leaf arrangement from flowering crowns with no guiding landmarks during dissection except the recurring more or less developed spathes. Many such dissections were made, inserting pins as each spathe was dissected out, so that the record so obtained could be preserved for comparison with others. These were then compared with idealized diagrams of the various natural arrangements, without obtaining absolute agreement in any case. Various fluctuations occur, probably due to torsion caused either by the

pressure of growth at the crown or by the subsequent great contraction of the tissues as they pass on into the less active portion of the trunk. This same contraction brings the fibrovascular system into such a compact and complicated tangle that any attempt to trace leaf arrangement through that channel is hopeless. We have in fig. 19, however, strong evidence in favor of the common 2–5 arrangement, at least during the monopodial stage. This specimen is apparently the result of a lateral bud coming into activity at considerable depth, and making greatly elongated growth quite unusual for the species in its effort to reach a suitable relationship to the surface. In the old trunk, after its normal contraction, it is impossible even to count leaf scars with any certainty, but here they are far apart, and very distinct until near the crown. A very small bud is to be seen in the lowest axil shown, then at intervals of five scars in each case are three more buds in succession, the compression of the crown making it impossible to follow the series any farther. These four buds, however, are so definitely superimposed in a vertical line on the trunk as to leave no doubt of the arrangement. Even in the crowded crowns, the 2–5 arrangement is approximated, the divergence being irregular, and, on studying cross-sections of lateral buds and embryos (figs. 3, 4), the leaf traces, although in such thick sections as to make actual measurement difficult, are very suggestive of the same arrangement. In view of this evidence, the conclusion is reached that *Spathyema* maintains the 2–5 phyllotaxy through its career from embryo to mature sympodial crowns.

There remains to consider one very interesting speculation concerning this plant. No one seems to know to what age it may attain, but like most slow growing plants it is evidently of very long life. Estimates of as much as seventy-five years have been made, presumably based on the length of trunk present, but in order to understand the uncertainty of any method of estimate we must consider the manner in which the plant counteracts the tendency of all vertical trunks to elongate into the air. This is partly taken care of by the contraction of the trunk itself, as already mentioned, but even so, with an addition of but a millimeter or two each year, and with only terminal growth like a palm, there must eventually be a mounting trunk, with its ultimate risk of being

blown over by wind, or as in the case of trees reaching a maximum height to which the vitalizing forces of the plant may be sent. This possible condition is avoided by *Spathyema* by sending from the crown each season a new circle of strong roots diagonally down to considerable depths, which when well anchored contract their length and drag the whole plant downward with such force that eventually the lower end of an old plant is worn round and smooth like a potato by the thrust into the deeper soil. Thus year by year the crown is maintained at a level somewhat below the surface of the ground, the trunk, with its complement of lateral buds and marked by the scars of leaves that flourished scores of years ago, descends deeper and deeper into the mucky soil. While we may dig it up and determine by its length or by counting as nearly as possible the leaf scars the approximate number of years represented, it is obvious that what has been worn away at the bottom may have been many times greater than that which remains, and it then becomes apparent that *Spathyema* is of indefinite age.

Summary

1. In the District of Columbia *Spathyema foetida* may be found in bloom during moderately warm weather at any time from November to March or April. Each plant has but one blooming period per year, but while some bloom early and usually are frozen later, others under the same conditions of warmth remain unaffected and do not bloom until spring.

2. Growth of spathes is not by leaps and bounds, but is rather slow and continuous; apparently aided by the plant's own internal warmth.

3. Flower, foliage, fruit, and seeds may be seen in such a broken sequence that their relationship may not be very apparent.

4. *Spathyema* pursues a monopodial existence for an indefinite number of years until the first spathe appears, after which it is sympodial with a succession shoot bearing two leaves. The upper axillary bud always becomes the new terminal, while the lower one passes into reserve, developing only in case the terminal crown is destroyed.

5. During the monopodial stage the leaves are all rolled in the

same direction, each entirely outside the next succeeding leaf. On reaching the sympodial stage growth expansion is facilitated by the constant reversal of direction in which the leaves are rolled.

6. Seeds are without seed coats, and the almost buried plumule is indistinguishable from the axillary buds held in reserve.

7. From a large crown as many as 31 leaf units have been dissected. These would provide the full complement of leaves for nearly three years, since it is rare to find a plant with more than 11 leaves expanded in one season. For each second leaf there is a spathe, but more than half of these are ultimately suppressed, probably through the mechanics of growth pressure, with the result of lessening the number of fruits and so securing a proper balance with photosynthesis.

8. Leaf arrangement in this species has been a moot question up to the present time. It is here shown to be definitely 2–5 during the monopodial stage, and this ratio is probably maintained throughout.

9. *Spathyema*, possessing contractile roots that year by year drag it downward into the soil, thereby wearing away by friction the lower end of the trunk, leaves no possible means of even estimating its age, which must be looked upon as indefinite.

CHEVY CHASE, MD.

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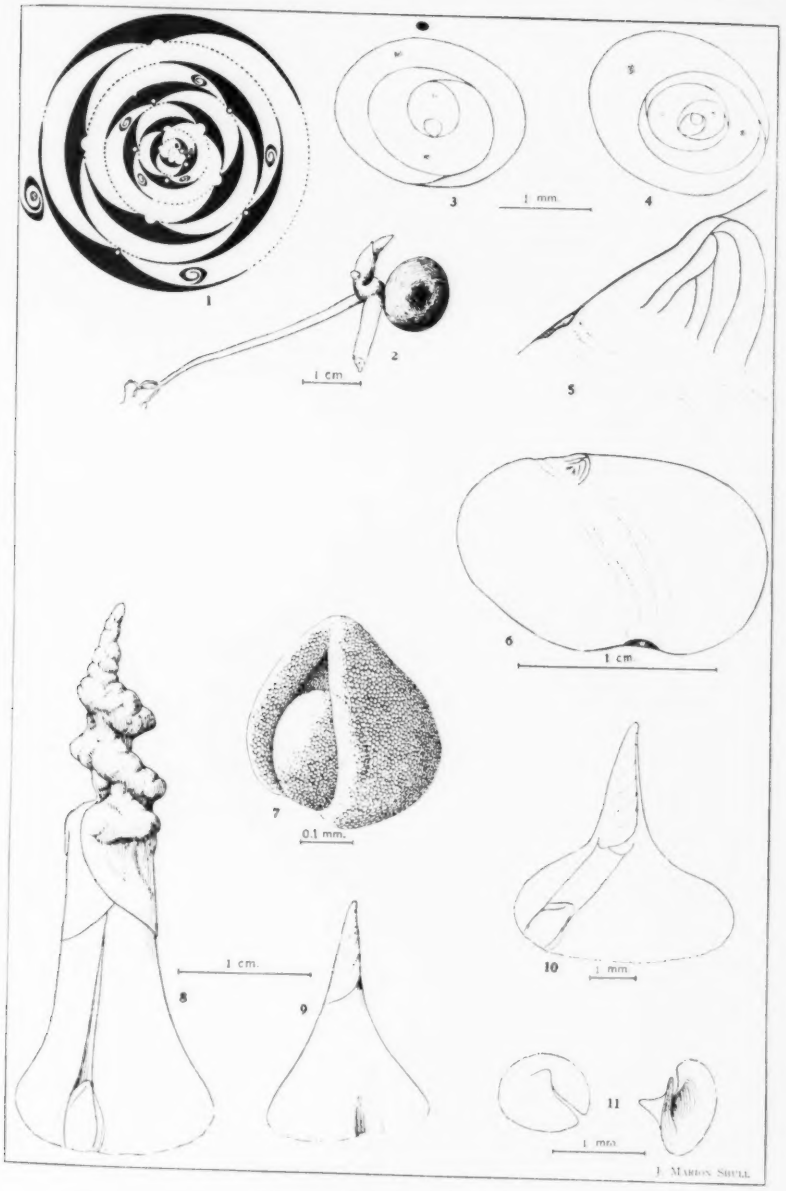
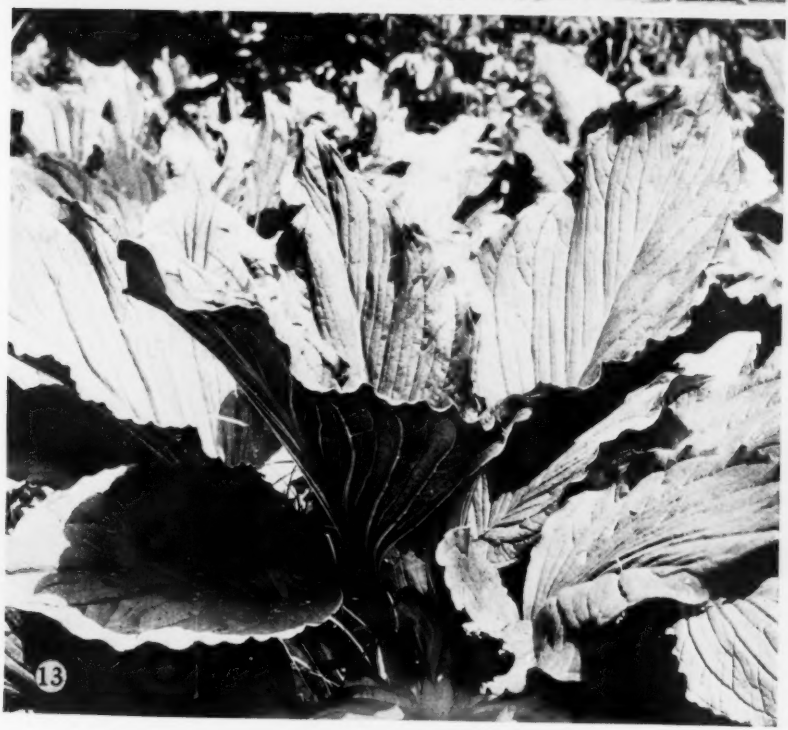
EXPLANATION OF PLATES I–IV

PLATE I

FIG. 1.—Diagram of leaf arrangement and spathe relationship in *Spathyema;* spathe being terminal bud, each successive circle represents



SHULL on SPATHYEMA



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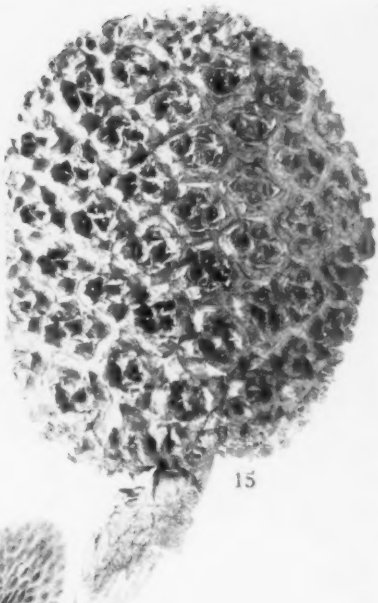
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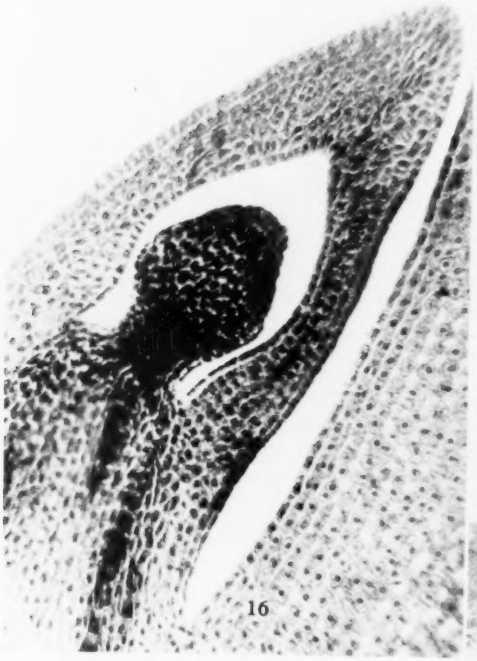
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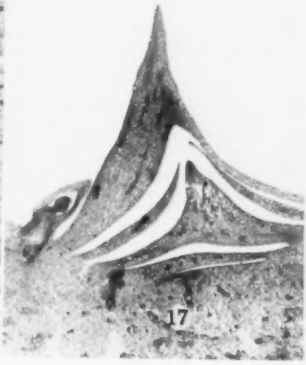
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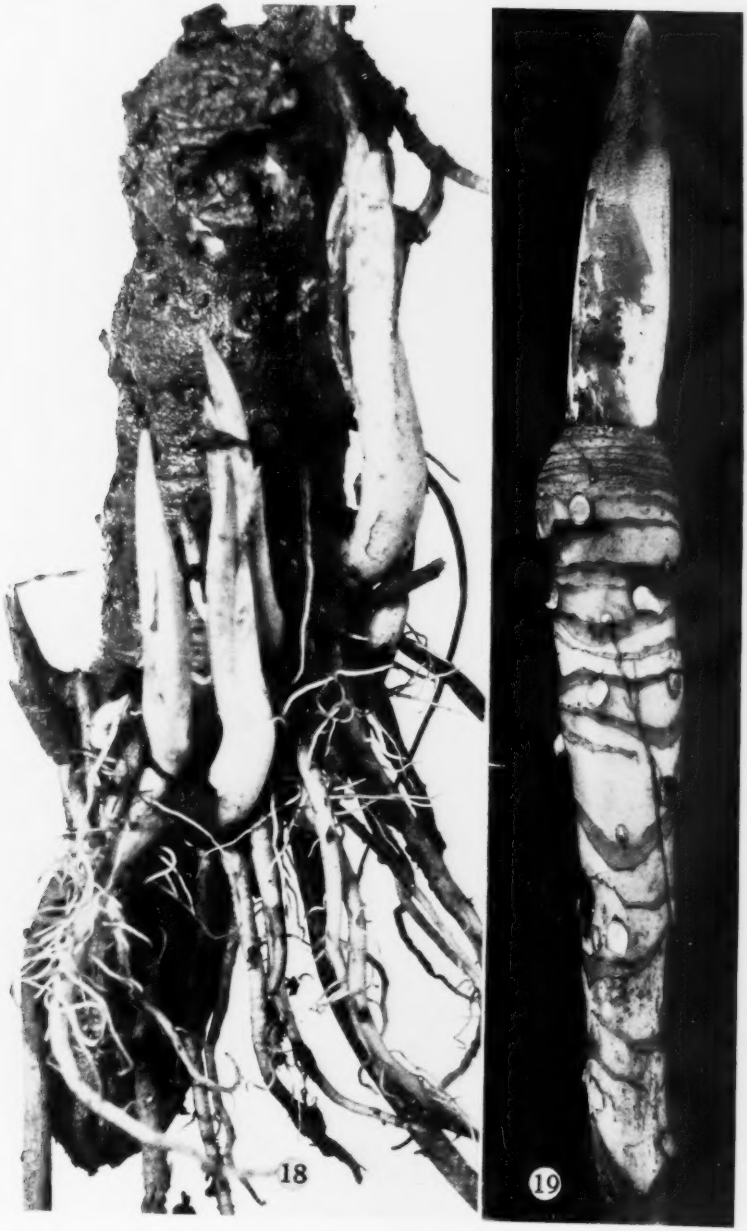
SHULL on SPATHYEMA



SHULL on SPATHYEMA

axillary bud lying between this and subtending leaf, missing in case of first spathe shown; each circle may also represent succession shoot of two leaves, lower one subtending a microscopic axillary bud that normally remains dormant, while axillary bud of upper leaf becomes, each in turn, new terminal destined to give rise to two leaves and then become a spathe.

FIG. 2.—One-year-old plant with seed still attached.

FIG. 3.—Axillary bud shown in cross-section.

FIG. 4.—Plumule as found in seed, shown in cross-section.

FIG. 5.—Plumule shown in longitudinal section.

FIG. 6.—Seed in longitudinal section, showing vascular relationship.

FIG. 7.—Last spathe dissected out, apparently not due to bloom until 27 months later (camera lucida).

FIG. 8.—Twelfth leaf unit dissected from vegetative bud in November, last destined to full development during succeeding spring season; probably spathe at base of leaf would have bloomed 15 months later, all those immediately preceding it being aborted.

FIG. 9.—Thirteenth leaf unit is rudimentary leaf which would close season's growth by developing into wintering-over sheath.

FIG. 10.—Seventeenth leaf unit from same bud.

FIG. 11.—Twenty-eighth leaf unit, now reduced to mere mammillary scale (camera lucida).

PLATE II

FIG. 12.—*Spathyema* plant March 6; about $\frac{1}{6}$ natural size.

FIG. 13.—Same plant, taken from same point but with different lens combination, May 21; about $\frac{1}{12}$ natural size.

PLATE III

FIGS. 14, 15.—Fruit in September; slightly reduced.

FIG. 16.—Spathe 27 months in advance of anthesis; ×120.

FIG. 17.—Center of crown with same spathe shown in fig. 16; ×14.

PLATE IV

FIG. 18.—Portion of old trunk freely giving rise to lateral growths from axillary buds held in reserve; natural size.

FIG. 19.—Elongated trunk of young plant, or rather lateral shoot, showing three distinct cycles of leaf scars and axillary buds that indicate 2–5 leaf arrangement; slightly reduced.

CROSSING IRIS PSEUDACORUS AND I. VERSICOLOR

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 330

M. LOUISE SAWYER

(WITH PLATES V–X AND ONE FIGURE)

In the progress of an experiment which was begun in the botanical garden at Grinnell College, Iowa, in the spring of 1918, the writer attempted to produce a hybrid between *Iris Pseudacorus* and *I. versicolor*. Reciprocal crosses were made, and, as has already been reported,[1] the progress was most promising. When the writer left Grinnell for the summer vacation, the ovaries of the cross pollinated flowers were distinctly larger than they were at the time of pollination, were apparently still growing and appeared vigorous, while the ovaries of flowers which had been castrated, covered, and not pollinated were shriveling, had a yellowish color, and were partly separated from their stalk by an abscission layer, if they had not already fallen off.

The promises of the June days were not fulfilled, however, for upon returning to the garden in early September, instead of the harvest of hybrid seeds on *I. Pseudacorus* that was hoped for, only a few withered pods were found still clinging to the plants. In addition to a number of wrinkled seeds, these pods yielded but one plump seed that appeared to be mature, and that one proved to be hollow. A similar attempt the following year had a similar outcome.

These results raised the question as to what occurs following pollination to so stimulate growth and to prolong the life of the ovary and yet fail to produce viable seed. The investigation here reported is concerned with that problem. Both the behavior of the pollen tube and the sequence of the stages in the developing ovules have been examined. The progress of development in ovules of cross pollinated flowers has been compared with that occurring in ovules of flowers which had been self pollinated.

[1] SAWYER, M. LOUISE, Hybridization in *Iris*. Iowa Acad. Sci. **28**: 1919.

I. Pseudacorus grows luxuriantly around the botany pond in Hull Court at the University of Chicago, and these plants were the source of most of the material used. Much of the collecting was done in the spring of 1920. The early spring of 1921 resulted in the flowering period ending before the writer could reach the University, and most of the 1922 collection is not yet cut.

In preparing to pollinate, buds were opened just enough to remove the stamens with forceps, and the inflorescences were then covered with bags of thin cloth. Such covering precluded the visits of bees, and the stigma was evidently sufficiently protected by its position. That this was true was indicated by the early death of those flowers which were not hand pollinated after being castrated and protected in this manner. Such flowers drop off in four to seven days after being covered.

The covered flowers which were to be crossed were kept under observation, and during the following day when the stigma was ripe, each flower was pollinated with *I. versicolor* pollen, a tag bearing the data as to the hour and date of pollination was attached, and the flower again covered. The protecting bag was left on until the flower had withered. Collections of the ovaries were made at intervals during the six weeks following pollination. The date of killing, after the early stages, was determined by the appearance of the ovary. It became evident that there was a gradual decrease in the rate of growth of the crossed flowers as compared with that of self pollinated flowers of the same age.

The striking difference in the two sorts of ovaries at the end of six weeks is shown in fig. 1. The ovaries of the crossed flowers were not only smaller, but they remained upright, while the larger and heavier fruits of the self pollinated flowers became pendent. They also differed in form. Three grooves run lengthwise of the crossed ovaries, with the line at the junction of the carpels at the bottom of the furrow, so that transverse sections (fig. 2) have three reentrant angles separating the locules. The transverse sections of the self pollinated ovaries are nearly terete (fig. 3). The walls of the crossed ovaries are drier and of a more papery texture than those of the selfed ovaries, and their locules in section are lenticular, while in the latter they are nearly circular. The difference in the external ap-

pearance of the ovules is still more significant. In the crossed ovaries the partly developed seeds are shriveled and of varying sizes, the largest not 1/100 of the volume of the selfed seeds. In the crossed ovaries examined at this age, no ovule showed promise of developing even the hollow seed shell which occasionally occurs.

FIG. 1.—Ovaries of *I. Pseudacorus* 6 weeks after pollination, showing smaller erect fruit resulting from cross pollinating with *I. versicolor* pollen, and heavier pendent fruit resulting when *I. Pseudacorus* pollen is used; photograph by SEDGWICK.

The collections were killed in chromo-acetic solution to which a little osmic acid was added (about 10 drops per 50 cc. of the killing fluid). They were imbedded in paraffin and cut in serial sections with a rotary microtome. For staining, both Haidenhain's iron-alum haematoxylin and the safranin-gentian violet combination were used. While both were satisfactory as stains, each had disadvantages. From the 8-nucleate embryo sac stage to the stage of endosperm tissue *Iris* ovule sections are difficult to handle. The contents of the embryo sac apparently soften when slides are in aqueous solutions, and during the long washing which is necessary after staining with iron-haematoxylin, they are frequently lost. On the other hand, the surface tensions which develop when slides are transferred from aqueous gentian to high grade alcohols have a similar effect. Even Land's fixative does not prove wholly satisfactory in overcoming these difficulties.

Behavior of pollen tubes

A study of the rate of pollen tube growth was made. Stalks bearing buds of *I. Pseudacorus* were cut and the stamens removed from

TABLE I

Flower	Pollen applied to stigmas of I. Pseudacorus	Time of pollination	Time of dissection	Time from pollination to dissection		Length of tubes
				Hr.	Min.	(mm)
I	×I. versicolor	9:20	10:20	1		0.05
	×I. versicolor	9:20	10:22	1		0.10
	×I. Pseudacorus	9:23	10:26	1		3.00
II	×I. versicolor	9:25	10:55	1	30	0.06
	×I. versicolor	9:25	1:25	4		5.00
	×I. Pseudacorus	9:27	1:30	4		7.00
III	×I. versicolor	9:30	5:00	7	30	7.00
	×I. versicolor	9:30	5:35	8	05	20.00
	×I. Pseudacorus	9:32	5:39	8		12.00
IV	×I. versicolor	9:35	5:10	7	30	14.00
	×I. versicolor	9:35	5:35	8		20.00
	×I. Pseudacorus	9:38	5:40	8		23.00
V	×I. versicolor	9:40	10:40	1		0.00
	×I. versicolor	9:40	4:40	7		8.00
	×I. Pseudacorus	9:42	4:45	7		23.00
VI	×I. versicolor	9:45	5:47	8		22.00
	×I. versicolor	9:45	5:50	8	5	24.00
	×I. Pseudacorus	9:48	5:58	8		24.00
VII	×I. versicolor	9:50	1:10	3	20	8.00
	×I. versicolor	9:50	1:15	3	25	5.00
	×I. Pseudacorus	9:52	1:20	3	28	15.00
VIII	×I. versicolor	10:00	1:35	3	35	10.00
	×I. versicolor	10:05	1:37	3	32	8.00
	×I. Pseudacorus	10:08	1:41	3	33	24.00
IX	×I. versicolor	10:10	12:05	1	55	0.00
	×I. versicolor	10:11	3:10	5		0.00
	×I. Pseudacorus	10:14	12:10	2		0.00
X	×I. versicolor	10:15	10:45		30	0.00
	×I. versicolor	10:15	11:15	1		0.06
	×I. Pseudacorus	10:18	11:48	1	30	0.10

the buds. The following day, as the stigmas ripened, two stigmas in each flower were pollinated with *I. versicolor* pollen, and to the third stigma *I. Pseudacorus* pollen was applied. At intervals the stigmas

were removed and the pollen tubes dissected out and measured. For each dissection the length recorded is that of the longest tubes.

Under the conditions of the experiment it is clearly not possible to have such uniformity of extraneous conditions as is desirable and essential to the securing of significant quantitative results. In this experiment one cannot be sure that all pollen grains from the same flower or even from the same anther have reached the same degree of maturity. Certainly no one can know that "ripe" stigmas of two different flowers, or two stigmas of the same flower, or even two parts of the same stigma are so nearly identical that a given pollen grain would develop tubes at exactly the same rate no matter to which of such stigmas it was applied. In view of these facts and the limited number of dissections which can be reported, the variations in results as indicated in table I are not surprising. Several sets of similar measurements were made, but they gave results so like those of this table that they are not presented.

Attention may be called to one conclusion based on these results. While there is a suggestion that *I. versicolor* pollen tubes tend to grow somewhat less rapidly on *I. Pseudacorus* stigmas than do *I. Psuedacorus* tubes, it is possible for conditions to be such that they grow with practically equal vigor (IV and VI, table I). In view of this fact and the fact that transverse sections of crossed ovaries show pollen tubes in the micropyle (figs. 5, 6), it is evidently not retarded growth of the pollen tubes which is responsible for the failure to secure a hybrid between these two plants.

Ovule

It was the plan to consider four phases in the development of the ovule: (1) rate of growth; (2) behavior of pollen tube; (3) development of endosperm; and (4) development of embryo.

1. RATE OF GROWTH.—An attempt was made to compare the rate of growth of the ovule in selfed and crossed material by measuring the length and breadth of the ovule and embryo sac. The necessity of using only similarly oriented ovules is evident. Only those cut in the median longitudinal plane were of use, which greatly limited the number of ovules available. Furthermore, it became evident, after some 200 measurements had been made, that the

amount of variation in size of ovules of the same age, especially from different ovaries, would make the results meaningless unless a very large number of ovaries of each age could be examined. Lack of sufficient material made it necessary to abandon this phase of the investigation.

2. POLLEN TUBES.—Pollen tubes were first detected in the micropyle 77–78 hours after pollination. The tube does not collapse after penetrating the micropyle, but holds its diameter in a rather remarkable way, both in selfed (fig. 4) and crossed material (fig. 5). It is very common for the tube to branch on reaching the nucellus; usually it merely forks (fig. 6), but occasionally it ends in three branches (fig. 4). HOFMEISTER[1] observed branched pollen tubes in two monocotyledons, *Panthos longifolia* and *Hippeastrum aulicum*. The latter species is one of the Amaryllidaceae, so that this phenomenon has been reported in at least one species which is not far removed from *Iris*.

3. DEVELOPMENT OF ENDOSPERM.—Transverse sections of ovaries killed at the stage when stamens should be removed, if the flower is to be cross pollinated, show the embryo sac not yet fully mature (fig. 7). The polar nuclei have not fused, nor are the antipodal cells organized. Fig. 8 from the antipodal end of an embryo sac, 12 hours after pollination, shows well developed antipodal cells, but the polars are not yet in contact. On the other hand, polars in contact were observed as early as 6 hours after (fig. 9) and as late as 54 hours after pollination (fig. 10). No difference between crossed and selfed ovules could be detected at these stages. The egg apparatus is then intact, and may be surrounded by numerous dark staining metaplasmic bodies, using that term to denote cytoplasmic inclusions. These bodies are probably starch, lying in the cytoplasm (fig. 11).

Each polar nucleus contains a conspicuous nucleolus. The nucleus formed by the fusion of polar nuclei at first shows two nucleoli (fig. 12), presumably one derived from each polar. Later a single large nucleolus is usual and is always the condition when the second male nucleus has arrived (figs. 14, 15). Cases like that shown in fig. 13 suggest that the single nucleolus is the result of fusing. At no

[1] HOFMEISTER, W., Neue Beitrage Zur Kenntniss der Embryobildung der Phanerogamen. Abhandl. Konigl. Sachs. Gesell. Wiss. **6**: 533–672. 1859.

time was polar fusion delayed as late as the time of the arrival of the male nucleus. This differs from the finding of GUIGNARD,[1] for he figures *Iris* sp. with the three nuclei in contact.

In crossed material the male nucleus was seen in contact with the nucleus resulting from the fusion of the polars at 77 hours after pollination (fig. 14). The same condition was observed in selfed material killed 78 hours after pollination (fig. 15). In both cases, in the same ovary with ovules showing fusion, there were ovules showing free endosperm nuclei, numbering two to four in crossed and running as high as eight in selfed material. As the selfed ovaries were a little in advance of the crossed ovaries, it is believed that the time difference is not of significance, and that the time of fusion is practically the same. The divisions in the endosperm of both types of material continue. Material taken 11 days after pollination shows the endosperm forming a peripheral layer of numerous nuclei still free (fig. 16). In selfed material the nuclei seem rather more closely packed, and it is believed that they are more numerous. Wall formation has begun by 13 days after pollination in both kinds of material.

As already stated, the egg may show a number of metaplasmic bodies, probably starch, imbedded in its cytoplasm (fig. 11). In self pollinated material such bodies appear abundantly in the embryo sac cytoplasm as well, at the time of fertilization (fig. 22). These were not seen in the crossed material, and this fact is the earliest indication of a difference in the nutritive content of the two endosperms. As time goes on the difference becomes pronounced. Selfed ovules develop an endosperm, which continues to carry a load of these metaplasmic bodies (fig. 20). Crossed material after 20 days shows in the occasional ovule which is still plump an endosperm tissue, not filling the cavity, and made up of thin walled cells with tenuous cytoplasm carrying few metaplasmic bodies, although their nuclei appear normal except for an unusual number of nucleoli (fig. 17). At 26 days the cavity of the ovule was filled with tissue, but it was like well advanced pith, the cells thin walled and almost without contents (fig. 18). In the more vigorous of the ovules, in those ovaries which were still living at 6 weeks after pollination the

[1] GUIGNARD, I., Sur les antherozoides et la double copulation sexual chez les vegetaux angiosperms. Compt. Rend. **128**: 864–871. 1899.

endosperm cells collapsed and formed a dark staining jacket around the embryo (fig. 43). Others at 6 weeks were like those described at 26 days. The extreme difference at 6 weeks between the endosperms of cross and self pollinated material is indicated by figs. 19 and 20.

4. DEVELOPMENT OF EMBRYO.—The material which yielded sections showing the second male nucleus in contact with the nucleus formed from the fused polars also demonstrated fertilization. Sperms in contact with the egg were seen earliest in material taken 76–78 hours after pollination (figs. 21, 22). This is in general agreement with the fertilization time in selfed *I. versicolor*. In that species the writer[1] reported male nuclei in the embryo sac 72 hours after pollination. Ovaries of this age show some ovules which give no evidence of fertilization, not even of pollen tubes in the micropyle; and other ovules, as has been said, may have two, four, or eight free endosperm nuclei. In crossed material the male nucleus was also seen in contact with the egg as late as 96 hours after pollination (fig. 23). Whether fertilization finally occurs in such cases cannot be stated. No evidence of disorganization at this stage was detected.

The cytology of fertilization is still under investigation. At present the writer would only call attention to the fact that a section of a one celled body in crossed material shows a nucleus with chromosomes in pairs (fig. 24). This arrangement and the number (which is approximately 24) suggest that fusion of the gametes does occur. A corresponding stage has not been seen in self pollinated material. In the crossed material, some of the ovules having gametes in contact still have an undivided primary endosperm nucleus, while others in the same ovary show as many as sixteen free endosperm nuclei. It is apparent that the fusion of the gametes is not so promptly consummated as is the fusion which completes the primary endosperm nucleus.

One- and two-celled embryo stages are found in crossed material, taken 6 days after pollination. The one-celled embryos differ from each other in appearance, as do those of two cells. As one-celled bodies persist in ovules at 7 (fig. 25), 9 (fig. 26), 11 (fig. 27), and 13 days (fig. 28) after pollination, occurring in the same ovaries as

[1] SAWYER, M. LOUISE, Pollen tube and spermatogenesis in *Iris*. BOT. GAZ. **64**: 159–164. 1917.

ovules with embryos of several cells, these differences are probably related to the differing destinies of these structures believed to be one-celled embryos. Among them some are found in which it is possible to recognize signs of disorganization. In such cases the nuclei are irregular in outline, as if the nuclear membrane were dissolving, and the rather structureless mass tends to stain heavily (figs. 26, 29).

In self pollinated material the two-celled embryo was not found in collections made earlier than the seventh day after pollination. At this time these ovules have a well developed layer of free endosperm nuclei. This may be one of the significant factors in the subsequent history. In the selfed ovules the embryos soon overcome the slight handicap of a day's delay at the start, and for a time their development runs parallel with the more vigorous embryos in ovules of the crossed material. In both types of material, successive cell divisions result in a row of three (fig. 30) or four (fig. 31) cells, after which the end cell undergoes a longitudinal division (figs. 32, 33). A second longitudinal division may occur, as is shown by the fact that a transverse section of the end of the embryo at this stage shows four cells (fig. 34). Elongation of the embryo may continue (fig. 35), or by divisions in the lower cells it may become more massive (figs. 36, 37, 39).

In self pollinated material the development of the embryo then begins to forge ahead at such a rate that the embryo is fully formed and the ovule has attained the ultimate size of the seed at the end of 6 weeks. In ovules of cross pollinated material the history is quite different. Death of the embryo may occur at any stage. A dying embryo 6 days after pollination is shown in fig. 29; others at 11 days in figs. 40 and 41. In these cases the endosperm was still in the free nuclear stage. Twenty-six days after pollination some of the ovules in ovaries that are still living have so shriveled as to nearly obliterate the almost empty embryo sac cavity. Others less withered are partly filled with endosperm, which may be still nucleated, or its cells may have lost their contents. An embryo of this age is shown in fig. 42 with its cells plasmolyzed and distorted. The endosperm surrounding it has reached the pithlike stage (fig. 18). Crossed material which was killed 6 weeks after cross pollination yielded some interesting sections. Most of the ovules were so hardened and shriveled that they infiltrated poorly, and usually no sign of the embryo could be

detected among the fragments of the sectioned ovules; perhaps they would not have been recognizable in any case. In one of the more favorable sections of a less distorted ovule a strand of contracted endosperm cells inclosed a section of the embryo (fig. 43). A few ovules had not hardened their coats. In one of these, which was but little wrinkled, the embryo was found (fig. 44). The endosperm which surrounded it and filled the ovule cavity, however, was pith-like (fig. 19), and it is believed that the embryo could not have lived much longer with so meager a source of food.

This most advanced embryo found in cross pollinated material is but a feeble dwarf, when compared with those of the same age resulting from self pollination. This embryo, consisting of a little mass of undifferentiated cells at the end of a short suspensor, measured 235 μ in length, 112 μ in width, and was contained in an ovule which was about 2500 μ long and 1250 μ wide. At the same age, that is, 6 weeks after self pollination, those ovules had become seeds 7 mm. in diameter and 3 mm. thick, and contained fully differentiated embryos which were 3.5 mm. long and about 0.3 mm. thick. Figs. 45 and 46, drawn to the same scale, show the relative sizes of the two contrasted embryos. The cells do not differ greatly in size (figs. 47, 48), although the cytoplasm in the crossed embryo was more attenuated, and lacked the metaplasmic bodies similar to those noted in the cytoplasm of the endosperm earlier in this paper. Whatever causes the delay in development, therefore, retards cell division. These embryos never get beyond the proembryo stage.

Conclusions

1. Castrated flowers of *I. Pseudacorus*, covered and not pollinated, drop their ovaries in 4–7 days after being covered.

2. Pollination of *I. Pseudacorus* with *I. versicolor* pollen stimulates the growth of the ovary and prolongs its life.

3. During this investigation such pollination has not resulted in viable seed.

4. Dissection of pollen tubes established the fact that the growth of *I. versicolor* pollen tubes on *I. Pseudacorus* stigmas is able to keep pace with the growth of *I. Pseudacorus* tubes on their own stigmas.

5. Sections show that the time required for pollen tubes to enter the micropyle is essentially the same in cross and self pollinated

ovaries. Tubes were seen in the micropyle of material killed about 77 hours after pollination.

6. The failure to secure viable seed was not due to a failure of the pollen tube to reach the ovules.

7. Free endosperm nuclei appear in both self and cross pollinated specimens shortly after the entrance of the pollen tube in both self and cross pollinated specimens.

8. Male nuclei were observed in contact with the egg in crossed as well as in selfed material.

9. In cross pollinated material the occurrence of the one-celled zygote showing approximately 24 chromosomes is believed to indicate that fusion follows.

10. Following fusion, the history of the zygotes resulting from cross pollination varies widely. Some die without division and death may occur at any subsequent stage.

11. After fertilization, the selfed zygote first lags behind the crossed zygote; then for a short time their development runs parallel; but soon the embryos produced by self pollination develop more rapidly than the crossed embryos. The most advanced embryo resulting from the cross was found in ovules killed 6 weeks after pollination. This embryo is but 2500 μ long and 1250 μ wide, while selfed ovules of the same age contain embryos 3.5 mm. long and 0.3 mm. thick, and are fully differentiated.

12. The endosperm in crossed material at 6 weeks after pollination is either a contracted strand or a pithlike tissue of cells without contents, while the endosperm in the selfed material consists of nucleated cells whose cytoplasm is crowded with metaplasmic bodies, food granules.

13. The failure to secure viable seed following cross pollination of *I. Pseudacorus* by *I. versicolor* is due to the death of the embryo at an early stage of development, although it may live long enough to have reached maturity.

The writer gratefully acknowledges the helpful suggestions and criticisms of Professor CHARLES J. CHAMBERLAIN during the progress of this work.

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EXPLANATION OF PLATES V–X

Abbreviations: *a*, pollen tube; *b*, male nucleus; *c*, second male nucleus; *d*, egg nucleus; *e*, synergids; *f*, polar nucleus; *g*, primary endosperm nucleus; *h*, free endosperm nucleus; *i*, antipodal nucleus; *j*, antipodal cell; *k*, embryo; *l*, metaplasmic bodies; *m*, endosperm.

PLATE V

FIG. 2.—Transverse section of ovary 6 weeks after pollination; three reentrant angles separate locules, which are lens shaped in section; ×2.

FIG. 3.—Transverse section of ovary 6 weeks after self pollination; ×2.

FIG. 4.—Micropylar end of ovule 78 hours after self pollination, showing uncollapsed pollen tube which ends in three branches at nucellus; ×895.

FIG. 5.—Micropylar end of ovule 6 days after cross pollination; pollen tube in micropyle; remnant of synergid, zygote, and free endosperm nuclei in embryo sac; ×540.

FIG. 6.—Micropylar end of ovule 77 days after cross pollination; pollen tube forked on reaching nucellus; ×895.

PLATE VI

FIG. 7.—Embryo sac from flower at age when buds were opened for removing anthers; egg apparatus developed and polar nuclei approaching, but antipodal cells not yet organized; ×1610.

FIG. 8.—Antipodal end of embryo sac 12 hours after pollination; antipodal cells organized but polar cells not yet in contact ×1610.

FIG. 9.—Polar nuclei in contact 6 hours after pollination; ×980.

FIG. 10.—Polar nuclei in contact in cross pollinated material 54 hours after pollination; ×980.

FIG. 11.—Egg apparatus still intact 54 hours after pollination; ×2540.

FIG. 12.—Mature embryo sac; nucleus formed by fusion of polars shows two nucleoli; ×2540.

FIG. 13.—Nucleus formed by fusion of polars, showing nucleoli in contact as though fusing; ×1610.

FIG. 14.—Antipodal end of embryo sac with two antipodal cells and second male nucleus in contact, with nucleus formed by fusion of polars; 77 hours after cross pollination; ×2540.

PLATE VII

FIG. 15.—Male nucleus in contact with nucleus resulting from fusion of polars, 78 hours after self pollination; ×2540.

FIG. 16.—Free endosperm nuclei 11 days after cross pollination; ×1610.

FIG. 17.—Cells of endosperm tissue 20 days after cross pollination; ×895.

FIG. 18.—Cells of endosperm tissue 26 days after cross pollination; cells have lost their contents; ×895.

FIG. 19.—Cells of endosperm tissue 6 weeks after cross pollination; ×895.

FIG. 20.—Cells of endosperm tissue 6 weeks after self pollination; ×895.

FIG. 21.—Fertilization 77 hours after cross pollination; pollen tube extends well into embryo sac; ×2540.

PLATE VIII

FIG. 22.—Fertilization 78 hours after self pollination; free endosperm nucleus shown lying in embryo sac cytoplasm which is filled with metaplasmic bodies; expanded end of pollen tube lies within embryo sac; ×2540.

FIG. 23.—Male nucleus in contact with egg 96 hours after cross pollination; ×2540.

FIG. 24.—Nucleus of zygote 6 days after pollination, with approximately 24 chromosomes mostly arranged in pairs; ×2540.

FIG. 25.—Zygote 7 days after cross pollination; ×2540.

FIG. 26.—Zygote 9 days after cross pollination; ×2540.

FIG. 27.—Zygote 11 days after cross pollination; ×2540.

FIG. 28.—Zygote 13 days after cross pollination; ×2540.

FIG. 29.—Two-celled embryo beginning to disorganize 6 days after pollination; membrane of inner cell broken down; ×2540.

FIG. 30.—Embryo 9 days after self pollination; ×980.

FIG. 31.—Embryo 9 days after cross pollination; ×980.

FIG. 32.—Embryo 9 days after cross pollination, having vertical division of terminal cell; ×980.

PLATE IX

FIG. 33.—Embryo in which vertical division occurred in fourth cell; ×980.

FIG. 34.—Transverse section of tip of embryo, showing 4 cells; ×2540.

FIG. 35.—Elongated embryo 11 days after cross pollination; ×540.

FIG. 36.—Massive embryo 9 days after cross pollination; ×540.

FIG. 37.—Massive embryo 11 days after self pollination; ×980.

FIG. 38.—Two-celled embryo 6 days after cross pollination; ×980.

FIG. 39.—Massive embryo 13 days after cross pollination; ×980.

FIG. 40.—Embryo beginning to die; nuclei of some cells disorganized (11 days after cross pollination); ×980.

FIG. 41.—Embryo dying; cells contracted and formless (11 days after cross pollination); ×980.

PLATE X

FIG. 42.—Embryo 26 days after cross pollination; ×980.

FIG. 43.—Strands of contracted endosperm inclosing embryo, 26 days after cross pollination; ×895.

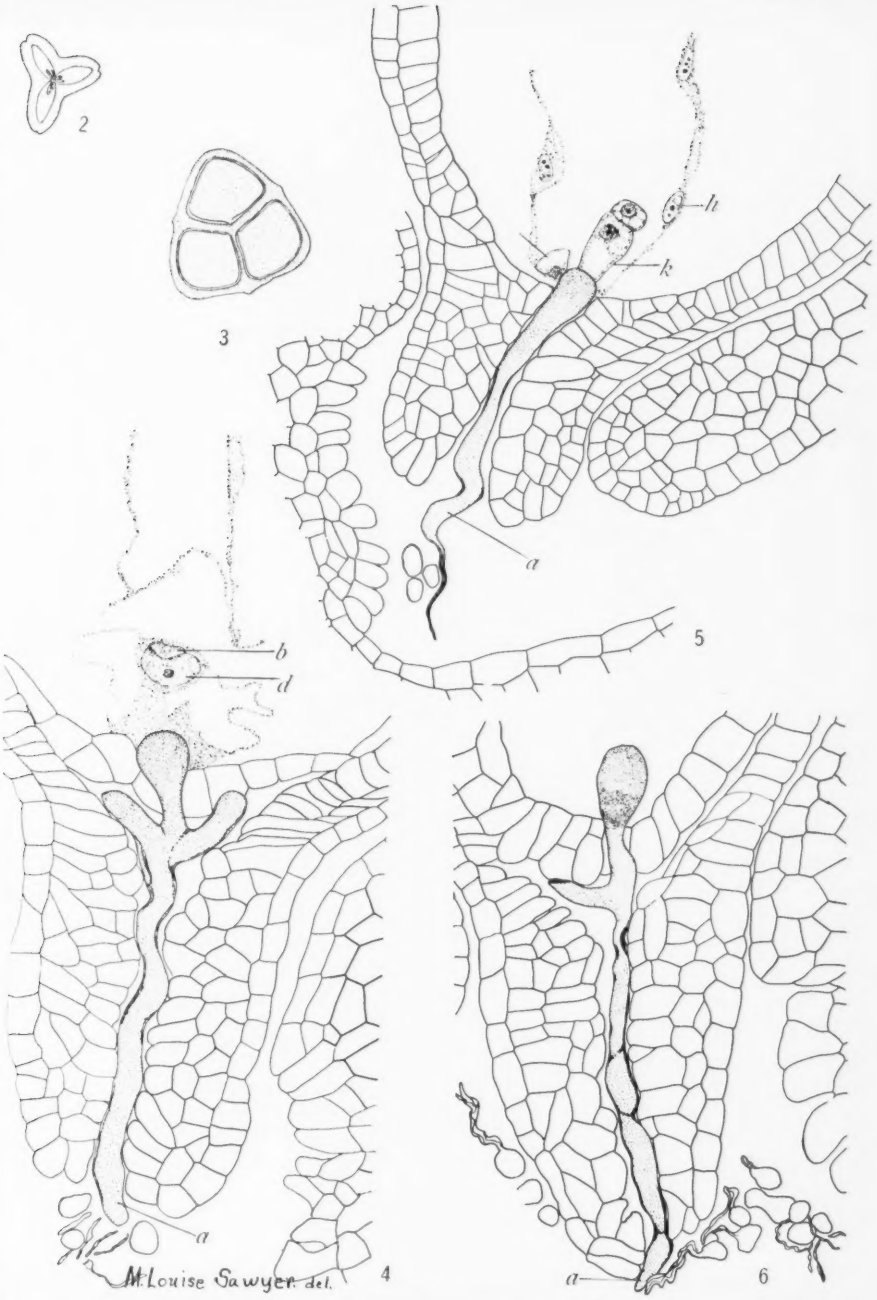
FIG. 44.—Embryo 6 weeks after cross pollination; ×895.

FIG. 45.—Embryo 6 weeks after cross pollination (same embryo as in fig. 44 drawn to same scale as next figure, for purpose of comparison).

FIG. 46.—Embryo 6 weeks after self pollination (drawn to same scale as fig. 45).

FIG. 47.—Group of cells from embryo 6 weeks after cross pollination; ×1610.

FIG. 48.—Group of cells from embryo 6 weeks after self pollination; ×1610.



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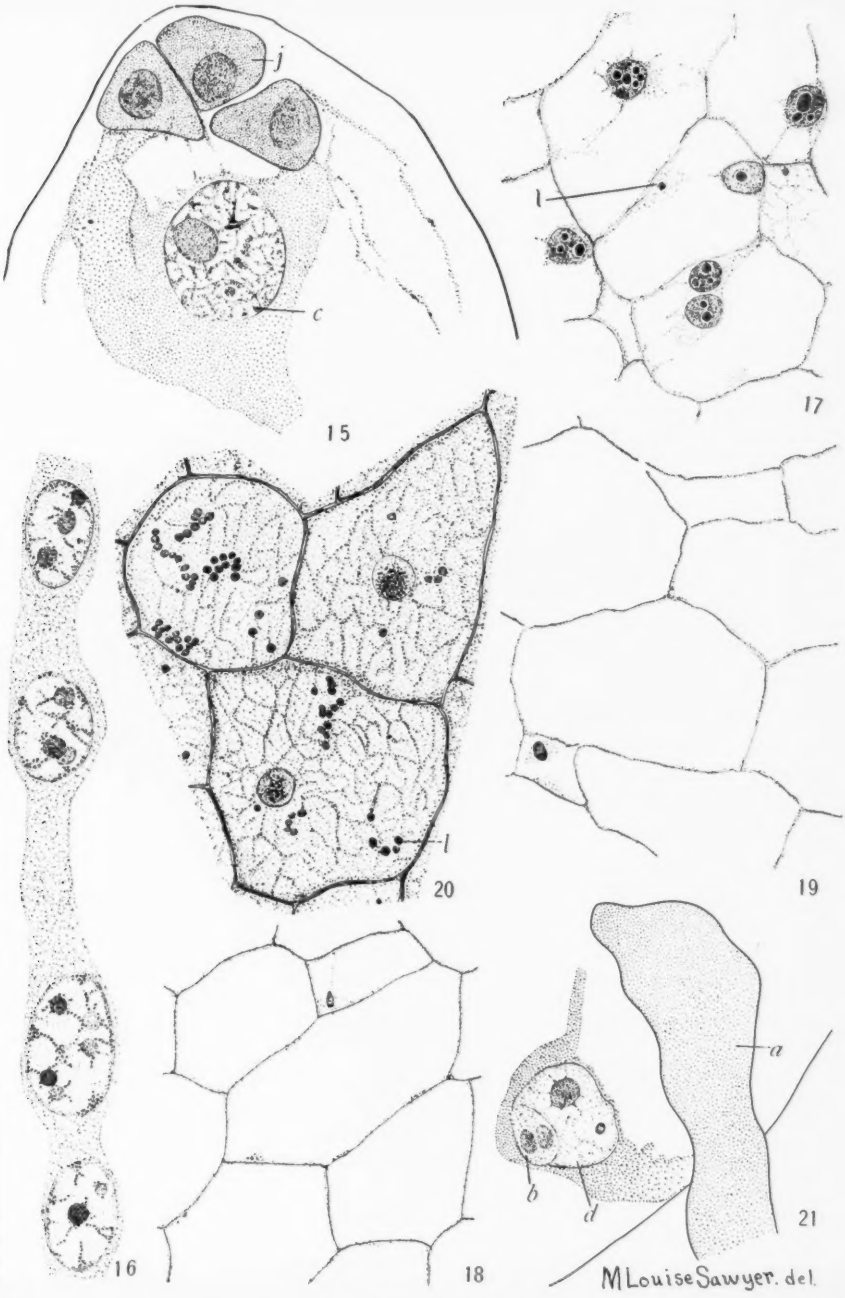

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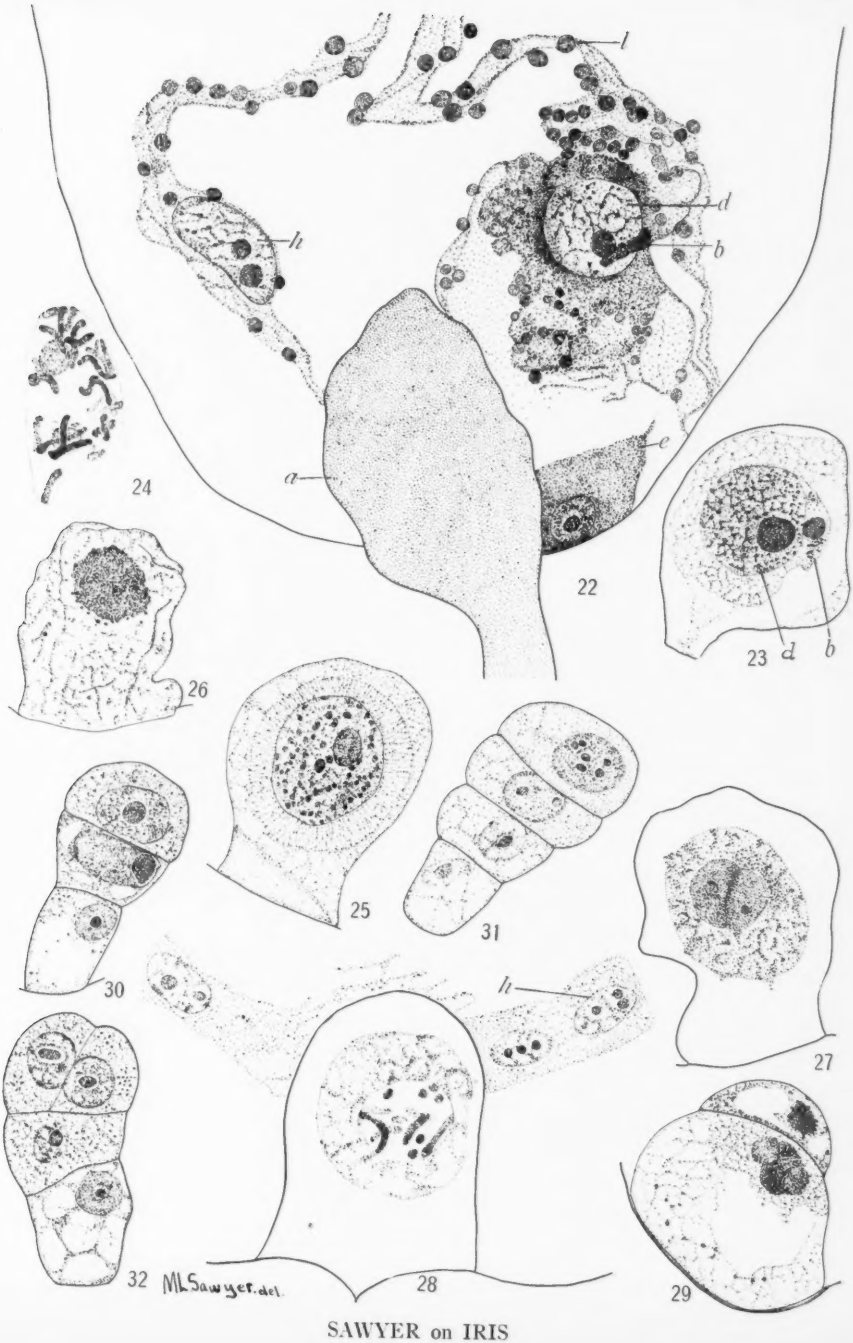

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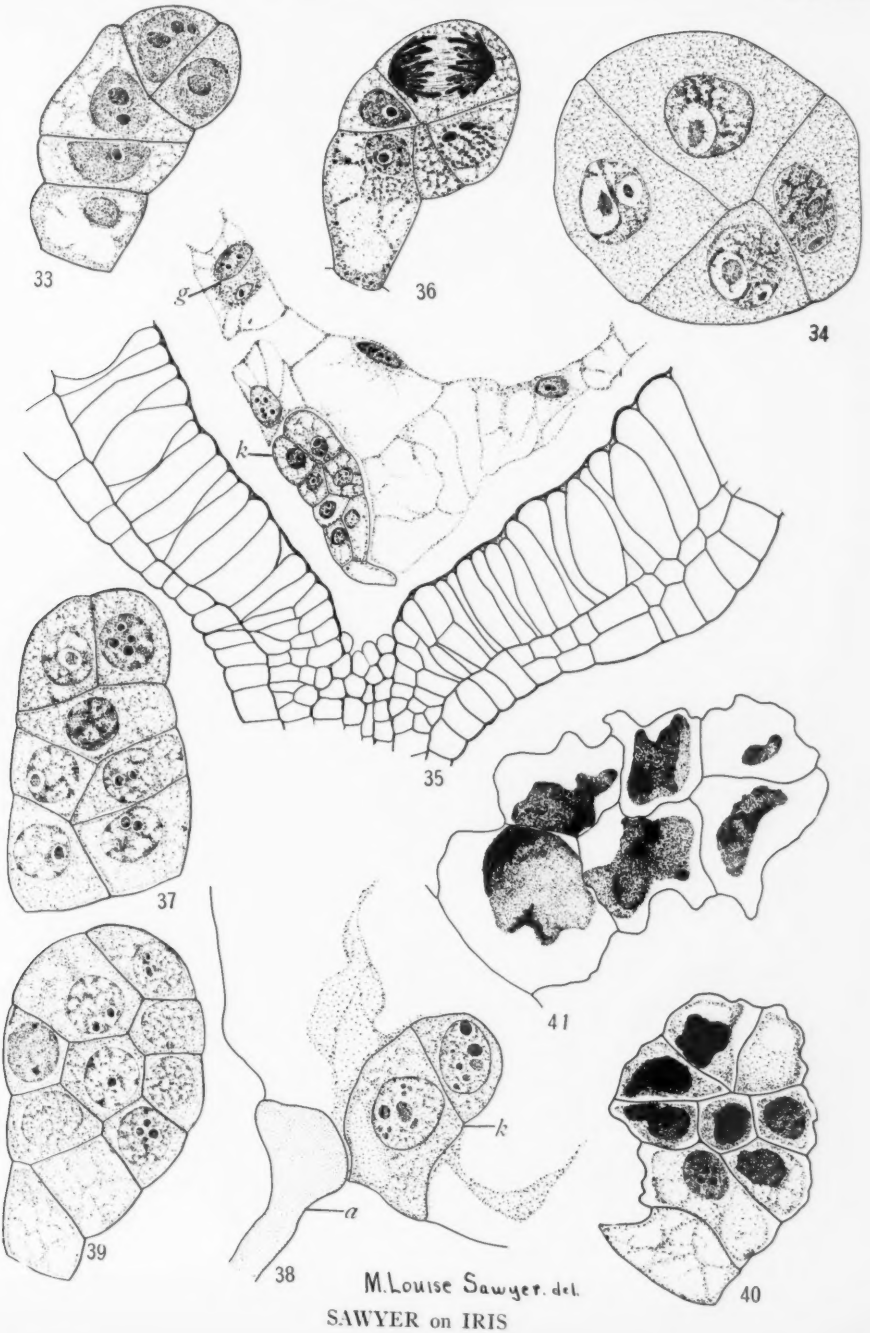

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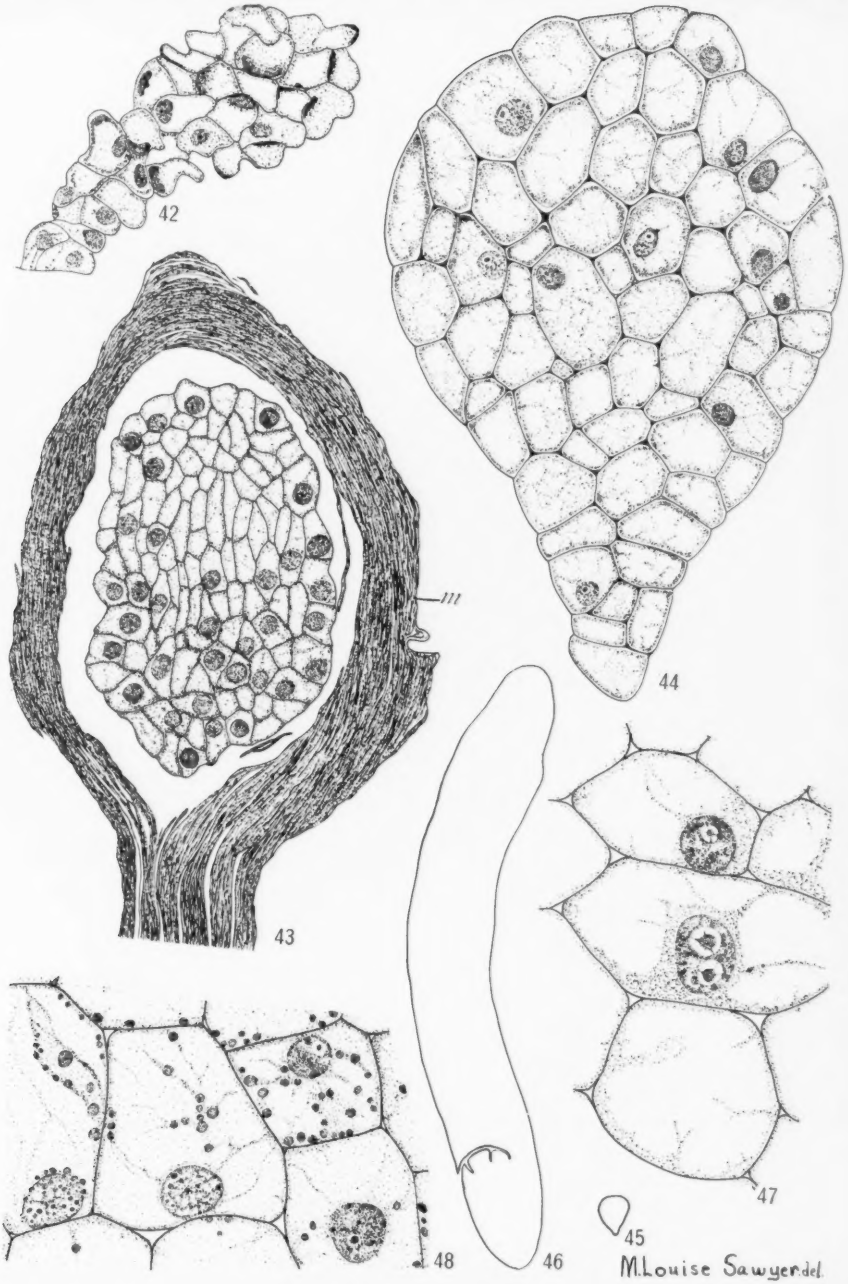

SAWYER on IRIS

CATALASE ACTIVITY AND THE AEROBIC AND ANAEROBIC GERMINATION OF RICE

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 331

TOSHITARO MORINAGA

Introduction

The relation of anaerobiosis and catalase activity has been studied by a number of workers, with somewhat conflicting results. According to RYWOSCH and RYWOSCH (**13**) and SPIRO (**16**), although aerobic bacteria contain much catalase, the anaerobic bacteria possess practically no catalase activity. The same claim has been made recently by MCLEOD and GORDON (**10**), and also by CALLOW (**4**). Some years ago LESSER (**7, 8**) showed that the blood of poikilothermous animals contains much less catalase than that of mammals. Among the animals which he investigated, *Ascaris* showed the least catalase activity. On the other hand, LOEW (**9**) found that yeasts and the bacillus of black-leg, an obligate anaerobe, possessed catalase activity, and stated that catalase might be an agent for fermentative as well as respiratory phenomena. Studying zymin in wheat seedlings, PREOBRASCHENSKY (**12**) found that substances which promote alcoholic fermentation also increase the amount of catalase, while inhibitors of fermentation also depress the catalase activity of the yeast. His conclusion was that catalase may take part in anaerobic processes. Notwithstanding the numerous contributions which have been made in recent years by both animal and plant physiologists, the physiological meaning of catalase activity is still very obscure, and we are not certain what part if any it plays in the dynamics of cell life, or whether it is connected with fermentative or respiratory processes.

The work reported here was undertaken to determine the influence of anaerobic and aerobic conditions of germination upon the development of catalase activity in seedlings of some of the higher green plants, with the hope that it might throw some light on the relation of catalase to the dynamic cell processes.

Materials and methods

A number of the grains were chosen for this work, including rice, because the latter, as has long been known, can germinate normally under water, or even in complete absence of oxygen. For comparison, seeds of barley, wheat, oats, and rye, which cannot be germinated so successfully in absence of oxygen, were used. Two varieties of rice, Ishijiro and Kagamochi, were obtained from Japan, and two others, Honduras and Acadia, from the Rice Experiment Station of Louisiana. Marquis spring wheat and seeds of barley and oats were supplied by the Wisconsin Agricultural Experiment Station. The rye was purchased from the Vaughan Seed Company of Chicago. All of these seeds were supposed to have been harvested in 1922. The viability tests showed that all of them, but particularly the varieties of rice, still retained high germinating power. The writer wishes to thank those who assisted by sending seeds of known age.

For the determination of catalase activity, a modified Appleman apparatus (**2**) was used, and the liberation of oxygen from hydrogen peroxide was used as a measure of the activity. The method was similar to that used by CROCKER and HARRINGTON (**6**). The Oakland Chemical Company "dioxogen" was used, the concentration of the H_2O_2 being determined for each bottle by permanganate titration, and the equivalent of 10 cc. taken for each determination. The acidity was neutralized by the addition of N/10 NaOH just before using.

Dry seeds were pulverized in a mortar and sifted through a 100 mesh sieve. Any portions not passing through the sieve were reground and sifted until there was no remaining part. The powder was thoroughly mixed in the mortar again, and samples weighed out from the mixed materials. When wet determinations were made, the materials were ground with sand and $CaCO_3$ in a mortar for five minutes. In the case of either wet or dry determinations, the material was put into the shaker bottle, with 25 cc. of distilled water, and allowed to reach the temperature of the bath. Then the equivalent of 10 cc. of the peroxide previously diluted to 25 cc. was added, after which the shaker was run at the rate of 100 times per minute, in a bath kept at 21° C. This temperature was adopted because it could easily be maintained; but catalase, like many other enzymes, is most active at about 35° C.

To determine the respiration occurring during germination when such tests were run, a bottle of known internal volume, containing a seed holder and a small NaOH receiver, was connected to a manometer. Several bottles of this type were used in a Freas thermostat at 25° C. The oxygen consumption was calculated from the reduction of manometric pressure, after the manner of SHERMAN (**14**), while the CO_2 released was estimated from the NaOH in the respirometer by the double titration method of BROWN and ESCOMBE (**3**). Blank experiments were also run to check the results.

Experimentation

1. COMPARATIVE CATALASE ACTIVITY OF AIR DRIED GRAINS.—To determine the catalase activity of the ungerminated dry seeds, 0.2 gm. of the dry powder of each kind of seed was taken. Table I shows the results for all the varieties of seeds used, including the four varieties of rice. It is seen that the rice varieties stand in contrast to the other grains in catalase activity of the dry, ungerminated seeds.

TABLE I

CATALASE ACTIVITY OF 0.2 GM. DRY SEED POWDER

SEED USED	OXYGEN LIBERATED IN CC. AFTER	
	6 minutes	16 minutes
Wheat	4.0	8.3
Oats	5.2	11.9
Barley	4.0	9.5
Rye	9.9	16.3
Rice (Ishijiro)	0.4	1.1
(Honduras)	0.2	0.6
(Acadia)	0.6	1.1
(Kagamochi)	0.5	1.4

2. COMPARATIVE CATALASE ACTIVITY IN AEROBICALLY GERMINATED SEEDS.—In this experiment, grains of oats, wheat, barley, and rice were germinated on moist filters in Petri dishes. One group of seeds was kept at about 25° C., floating on the water bath, and another at 16° C. in an icebox. The seeds were tested at equal stages in germination, the rice requiring considerably longer time to reach a given stage of development. Much more time was required at the lower temperature than at the higher one, for a particular stage to

be reached. Ten of the seeds were used for each determination. The results are shown in table II.

3. CATALASE ACTIVITY IN ANAEROBICALLY GERMINATED RICE.—The rice was germinated in thoroughly boiled distilled water, protected from contact with air by a mercury seal. This method was checked by germinating the seeds in purified hydrogen gas. The rice (Ishijiro) germinated under these conditions in two days, although it did not develop roots. The samples, ten grains or seedlings of each,

TABLE II

CATALASE ACTIVITY OF AEROBICALLY GERMINATED SEEDS; OXYGEN IN CC. AT STANDARD CONDITIONS

MATERIAL	HOURS GERMINATED	TEMPERATURE (° C.)	OXYGEN LIBERATED	
			6 minutes	12 minutes
Oats	18	25	13.5	23.1
Wheat	24	25	18.4	27.0
Barley	24	25	14.9	23.3
Rice (Ishijiro)	54	25	10.8	18.2
Oats	41	16	15.6	24.6
Wheat	46	16	15.4	14.0
Barley	48	16	12.6	21.7
Rice (Ishijiro)	258	16	9.6	16.8

were taken from the germinators each day for catalase tests. Comparative tests were made on grains growing under aerobic conditions on wet filter papers, and with seeds growing under 7.7 cm. of water, unboiled and unsealed. Note was made of the average state of development of plumule and radicle under the conditions employed. The results of these experiments are given in table III.

4. CATALASE ACTIVITY OF RICE GERMINATED AT REDUCED OXYGEN PRESSURES.—In this experiment the rice was germinated under reduced atmospheric pressure, as follows: $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, and $\frac{1}{16}$ atmospheres. The samples, ten grains or seedlings of each, were taken each day for a catalase determination. The results are shown in table IV.

The reduction of catalase activity is very marked as the oxygen pressure is reduced.

5. CATALASE ACTIVITY OF RICE GERMINATED UNDER WATER AT DIFFERENT DEPTHS.—Ordinary water of 2, 4, 6, 8, 15, and 30 mm. depth was kept in Petri dishes and beakers in which Kagamochi

TABLE III

CATALASE ACTIVITY OF RICE GROWN ANAEROBICALLY; OXYGEN IN CC. AT STANDARD CONDITIONS

CONDITION OF GERMINATION	TIME IN HOURS	OXYGEN LIBERATED		DEVELOPMENT IN MM.	
		6 minutes	12 minutes	Plumule	Radicle
Wet filters	24	2.7	4.5	0	0
	48	12.6	19.7	Starting	2.0
	72	21.9	32.2	2.8	9.8
	96	35.2	46.7	5.2	22.9
Under water 7.7 cm.	24	1.1	2.4	0	0
	48	2.9	5.4	2.0	0
	72	5.3	9.5	10.0	Starting
	96	8.2	12.9	19.0	3.0
In hydrogen gas	24	1.1	2.0	0	0
	48	1.4	3.0	1.0	0
	72	2.1	4.2	4.8	0
	96	2.5	4.6	15.3	0
In boiled distilled water under seal	24	0.9	2.2	0	0
	48	1.1	2.0	1.0	0
	72	1.4	3.2	6.0	0
	96	1.4	2.6	9.0	0

TABLE IV

REDUCED OXYGEN PRESSURE AND CATALASE; GERMINATION AT ROOM TEMPERATURE

ATMOSPHERIC CONDITION	HOURS OF GERMINATION	OXYGEN LIBERATED		DEVELOPMENTAL STAGE, IN MM.	
		6 minutes	12 minutes	Plumule	Radicle
1/2	24	2.0	4.3	0	0
	48	8.5	13.7	Starting	Starting
	72	22.7	33.2	1.4	6.4
	96	33.7	47.0	5.6	19.6
1/4	24	0.9	2.5	0	0
	48	4.9	10.0	Starting	Starting
	72	12.5	21.4	1.5	3.9
	96	31.9	46.5	20.1	21.6
1/8	24	1.1	2.4	0	0
	48	3.0	5.9	Starting	Starting
	72	7.2	13.0	2.5	2.2
	96	13.4	22.6	6.2	11.1
1/16	24	1.1	2.1	0	0
	48	1.7	3.5	Starting	Starting
	72	3.2	6.4	4.7	0.8
	96	5.1	10.5	10.8	4.3

rice was germinated. After six days of submerged germination and growth, ten seedlings of each were taken as samples for the determinations. The results are tabulated in table V.

TABLE V

SUBMERGENCE AND CATALASE ACTIVITY OF RICE; GROWN AT ROOM TEMPERATURE

CONDITION	OXYGEN LIBERATED		STAGE OF DEVELOPMENT		RATIO, PLUMULE / RADICLE
	3 minutes	6 minutes	Plumule	Radicle	
On wet paper	33.9	46.8	7.8	16.0	0.48
In 2 mm. H_2O	28.9	40.8	9.5	21.0	0.45
In 4 mm. H_2O	26.1	37.7	10.5	22.0	0.47
In 5 mm. H_2O	16.3	26.8	18.0	6.8	2.64
In 8 mm. H_2O	14.7	23.0	17.8	4.3	4.13
In 15 mm. H_2O	10.8	17.4	13.2	3.3	4.00
In 30 mm. H_2O	10.4	16.9	14.0	4.0	3.50

About the same results were obtained with Ishijiro. The reduction of catalase activity of seedlings grown under water is very marked as the depth increases. The radicle development falls off rapidly with increasing depth of submergence.

TABLE VI

SUBMERGENCE AND CATALASE ACTIVITY OF WHEAT AND BARLEY; GROWN AT ROOM TEMPERATURE

PLANT	CONDITION	LENGTH OF PLUMULE (MM.)	OXYGEN LIBERATED	
			2 minutes	4 minutes
Wheat	On wet paper	29	47.5	68.4
	In 2 mm. H_2O	30	42.4	64.9
	In 4 mm. H_2O	20	26.3	49.7
	In 6 mm. H_2O	0	4.1	8.1
	In 8 mm. H_2O	0	3.0	5.8
Barley	On wet paper	26	37.8	61.3
	In 2 mm. H_2O	27	25.9	43.7
	In 4 mm. H_2O	19	24.3	42.0
	In 6 mm. H_2O	0	2.4	6.8
	In 8 mm. H_2O	0	2.6	6.3

6. CATALASE ACTIVITY OF WHEAT AND BARLEY GERMINATED IN SHALLOW WATER AT DIFFERENT DEPTHS.—These experiments were conducted as were those upon rice. After four days five seedlings or grains were taken for a determination, with the results shown in table VI.

The decrease in catalase activity is very great between 4 and 6 mm. in depth.

7. Decrease of catalase activity under anaerobic conditions.—The rice (Ishijiro) was put on moist paper for four days, and then transferred into the bottle containing thoroughly boiled distilled water. The samples, of ten seedlings each, were taken every second day for the catalase tests. The results are tabulated in table VII.

TABLE VII

Decreasing catalase activity with anaerobiosis; standard conditions

Hours under water	Oxygen liberated		Growth under water (mm.)	
	6 minutes	12 minutes	Plumule	Radicle
0	43.1	53.9	0.0	0.0
48	30.8	48.8	4.3	2.8
96	26.8	42.0	6.6	3.1
144	18.3	27.2	12.0	0.5

8. Respiration of aerobic and anaerobic seedlings.—In this work forty grains of Ishijiro were kept on moist paper for aerobic respiration, and in boiled distilled water for anaerobic germination. They were allowed to respire under these conditions for two or four

TABLE VIII

Respiration of aerobic and anaerobic rice seedlings; oxygen and carbon dioxide in cc. at standard conditions

Time	Germination method	Oxygen absorbed	Carbon dioxide liberated	CO_2 / O_2	O_2 aerobic / O_2 anaerobic
2 days	Aerobic	4.9	5.8	1.17	
	Anaerobic	2.8	4.0	1.41	0.56
4 days	Aerobic	9.2	9.4	1.02	
	Anaerobic	4.1	4.3	1.05	0.44

days, after which the seedlings were transferred to the respirometers, the condition of temperature having been kept constant at 25° C. throughout the development and respiration tests. The oxygen taken in and the carbon dioxide given off in 20 hours were determined for each. The results are given in table VIII.

Then eighty seedlings, which had been grown under aerobic or anaerobic conditions for 4 days as before, were transferred to bottles in which air had been replaced by hydrogen. The carbon dioxide given off in 20 hours was determined for each, with the results presented in table IX.

TABLE IX

CARBON DIOXIDE LIBERATION BY AEROBIC AND ANAEROBIC SEEDLINGS IN HYDROGEN

Materials	Aerobic	Anaerobic
Ishijiro rice (80)	10.7	8.4

Discussion

From the first experiments it is clear that the catalase activity of the dry seeds of rice is only about one-tenth of that of barley, wheat, oats, and rye. In practice, rice is germinated under shallow water with a reduced supply of oxygen, and it is known that it can germinate in a medium which contains no free oxygen (**11, 17**). The same is true also of *Alisma Plantago aquatica* (**5**). It is considered a general characteristic of seeds which grow in media containing little free oxygen, to be able to germinate without it (**15**). As mentioned in the introduction, the anaerobic bacteria do not contain any catalase, or quantities so small as to be negligible, and *Ascaris* contains very little. Both live in oxygen free media. Although other experiments are needed on more diverse material to determine the facts, when rice is compared with the other grains, there seems to be some connection between the reduced demand for oxygen for germination and the reduced amount of catalase activity possessed by rice. Experiment no. 2 shows that all the kinds of grain examined except rice possess nearly the same catalase activity when they germinate on wet paper. There is also little difference in catalase activity when the seeds are germinated at different temperatures. The germinating rice contains about seven-tenths the activity of the others, but it was noticed that rice required longer for germination than the other seeds.

From the third set of experiments it is evident that the free oxygen content in the medium in which the rice is germinated affects very remarkably the increase of catalase activity during the course of

germination. In a medium of thoroughly boiled water the activity did not increase; that is, after 24 hours in the boiled water, ten grains liberated 2.21 cc. of oxygen in 12 minutes, whereas after 96 hours they liberated only 2.6 cc. of oxygen in 12 minutes. The average plumule development in this latter case was 9 mm. in length. In a medium of hydrogen gas the catalase activity increased very slightly, but this slight increase was supposed to be due to the insufficient replacement of the air by hydrogen. Under water of 7.7 cm. depth, or on wet paper in atmospheric conditions, the catalase activity increased very rapidly. Thus after 96 hours those in water liberated 12 cc. of oxygen in 12 minutes, and those on filter paper 46.7 cc. In each case ten seedlings were used.

The fourth experiment was carried out to verify by another method the relation between oxygen and catalase already mentioned. Under one-half atmospheric pressure, catalase activity increased rapidly, as under normal pressure, but with one-fourth atmosphere, development of activity was retarded for the first two or three days, although it increased very rapidly after that. Under one-eighth and one-sixteenth atmospheres, the increase of the catalase action was retarded corresponding to the decrease in pressure. As has been observed by others (**1, 9, 11**), the development of plumule and radicle is readily affected by the amount of free oxygen in the medium. Oxygen retards the elongation of the plumule and accelerates the development of the radicle. The lack of oxygen has exactly the reverse effect.

The data in tables V and VI show more closely the relation between water depth in the germinator and development of catalase activity. A little increase, say 2 mm., clearly affected the catalase development of the seedling. When the depth of water reached 6 mm., which is just enough to cover the whole caryopsis, catalase activity dropped rapidly, and then with increasing depths more slowly. Not only is catalase sensitive to the lack of oxygen, but also the plumule and radicle. Barley and wheat grains germinated in water of 2 mm. depth contained more catalase than those germinated under 4 mm. of water. They refused to germinate in water deeper than 6 mm., and showed very slight or no increase in catalase activity. According to THORNBER (**18**) they

do not retain vitality very long under these conditions. At this depth normal development of chlorophyll is interfered with. The plumules remain pale green, and in boiled water developed no chlorophyll at all. The catalase activity does not increase at greater depths, but actually decreases, even though growth may continue in the medium devoid of oxygen. In experiment no. 7, using ten seedlings, the catalase decreased from 53.9 cc. oxygen equivalent to 27.2 cc. equivalent in 144 hours. The average growth of plumules here was 12 mm.

As to normal respiration, the aerobic seedlings with much catalase, compared with anaerobic seedlings, took in about twice the volume of oxygen in 20 hours. The respiratory ratio was much greater than unity at the beginning of germination. This fact was more conspicuous for the anaerobic seedlings. These two kinds of seedlings, aerobic and anaerobic, gave off carbon dioxide in the ratio of 1:0.78 in anaerobic respiration; and the amount of carbon dioxide given off by the anaerobic seedlings in both kinds of respiration was nearly the same.

From the data as a whole, the writer concludes that there is a close relation between the amount of catalase activity in the seedling and the amount of free oxygen in the medium in which it germinated, and that catalase seems to have a close positive connection with the normal respiration, even if it seems to have some retarding effects, directly or indirectly, on the fermenting process estimated by the amount of carbon dioxide given off.

Summary

1. The amount of catalase in the dry seeds of rice is much less than that of wheat, barley, oats, and rye, being in fact about one-tenth as great.

2. Rice germinating aerobically contains much catalase, about seven-tenths as much as germinating wheat, barley, and oats.

3. The catalase does not increase in the course of anaerobic germination, but it does slowly in the course of germination in a medium which contains a reduced amount of oxygen. Hence the ratio of increase of catalase activity is a function of the free oxygen in the medium.

4. The free oxygen affects directly or indirectly the development of plumule and radicle, and also of the chlorophyll in it.

5. The catalase once increased by aerobic conditions decreases during anaerobiosis, while growth of the plant continues.

6. Aerobically grown seedlings with high catalase use much more oxygen than do anaerobically grown seedlings with low catalase activity. The anaerobic seedlings, however, give off a comparatively large amount of carbon dioxide during respiration, whether under normal aerobic or under anaerobic conditions.

The writer takes pleasure in expressing here his cordial thanks to Dr. CHARLES A. SHULL, with whom this work was undertaken, and to Dr. SCOTT V. EATON for their valuable suggestions and criticism given throughout the progress of the work.

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DIFFERENTIATION OF SPORANGIA IN MARSILIA QUADRIFOLIA

CONTRIBUTIONS FROM THE HULL BOTANICAL LABORATORY 332

CORNELIA C. MARSCHALL

(WITH NINE FIGURES)

Most of the stages in the development of the megasporangium of *Marsilia quadrifolia* have been described. It is the purpose of this paper to reexamine the work done by others, to add a few stages, but particularly to show where the differentiation of the sporangia occurs.

The material for this investigation was collected and kindly sent by Miss ETHEL THOMAS from Charleston, Illinois. Some of the material was fixed in chromoacetic acid with one cubic centimeter of osmic for every hundred cubic centimeters of the solution; and some was fixed in corrosive sublimate heated to 85°. The sections were cut 8–10 μ thick and stained with Haidenhains' iron-alum haematoxylin.

Historical

JOHNSON (2) determined that in each sorus the megasporangia are derived from a single megasporangial cell, and the microsporangia from two microsporangial cells formed in the basiscopic marginal cell of each soral segment. These megasporangial and microsporangial cells are sister cells. The microsporangial cells increase in number by radial growth and division. The megasporangium mother cells are pushed out by the growth of the plerome into the soral cavity far beyond the microsporangium mother cells. They swell laterally to several times their former size, and in so doing push the microsporangial cells around to a position nearly at right angles to their former one.

The megasporangium mother cell finally divides by three inclined walls to form the tetrahedral apical cell of the megasporangium. This apical cell cuts off two more segments on each of the three sides, which form a stalk and basal wall cells of the sporangium.

Then a pericline is formed near the outer end of the apical cell, cutting off the archesporium and completing the sporangium wall. While the microsporangial cells are being pushed aside, each has divided by anticlines approximately parallel to the segment wall, first to two, then to four. These come to lie parallel with the segments of the apical cell of the megasporangium. Of the four cells formed from each of the microsporangial cells, the lower three form sterile tissue of the placenta, while only the upper one, next to the megasporangium, actually forms microsporangia. Each of these upper cells divides by walls transverse to the axis of the sorus to form four cells of each side of each megasporangium. Then each of these four cells swells out to the placenta and divides into a basal cell and an outer cell, in which outer cell is formed later the tetrahedral apical cell giving rise to the stalk, wall, and archesporium.

According to CAMPBELL (1), the checking of the apical growth in the megasporangium results in a very short stalk; in the microsporangium the stalk is longer. The archesporial cells by a series of divisions give rise to a well developed tapetum of two and often three layers. At the same time sixteen sporogenous cells are formed. CAMPBELL also states that soon after the first divisions of the central cells are completed the cell walls of the tapetum begin to dissolve, but for a time the sporogenous cells remain together. Next they become isolated and round off for their final divisions into spores. In stained sections the nucleated protoplasm of the tapetal cells is very evident after the walls have disappeared. He states: "At this time the difference in the two sporangia becomes manifest. Those in the lower part of the sorus, that is the oldest ones, form megasporangia, the upper ones microsporangia."

The young spores of each tetrad show a marked tendency to hang together by strong protoplasmic strands, as was first shown by STRASBURGER (8). SHATTUCK (6) states: "These strands are the first recognizable morphological feature by which one can determine that a sporangium is to form a megaspore rather than microspores." These strands persist until the megaspore is quite mature, and in many instances can be seen on the papilla of the germinating megaspore, which still subtends the three members of the tetrad.

In the microsporangium every one of the sixteen mother cells produces four functioning microspores, sixty-four in all; while in the megasporangium only one spore out of sixty-four, all of which seem to be identical in every particular when the tetrands are first formed, produces a megaspore. A closer examination reveals the fact that this one megaspore does not gain ascendency without a struggle. In fact, many instances were found in which there was rivalry between two enlarging potential megaspores. Yet in the end, one centrally placed spore always gains the ascendency and the others disintegrate.

Investigation

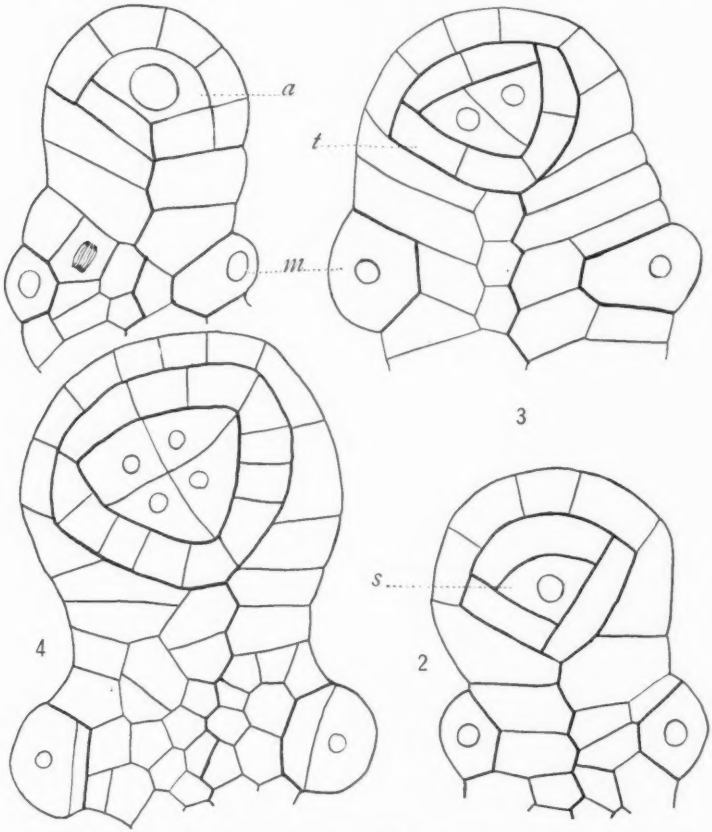
The early development of the megasporangium proceeds in the usual leptosporangiate manner, the central tetrahedral archesporial cell cutting off, by periclinal walls, the primary tapetal cells, which are easily distinguished from the outer cells by their more granular appearance. The outer cells divide by transverse divisions (figs. 1, 2).

The single sporogenous cell divides soon after the first tapetal cells have been cut off. The first division is usually transverse, but it may be more or less inclined or even vertical (fig. 3). Then, at right angles to this wall, two successive divisions occur, the first resulting in the quadrant stage, the second in the octant stage (fig. 4). These eight cells divide again, giving rise to the sixteen spore mother cells (fig. 5).

During the division of the sporogenous cells the tapetal cells divide by both anticlinal and periclinal walls, forming a tapetum of two or three layers of cells, a more extensive tissue than is found in the Polypodiaceae. The epidermal cells also divide by a number of transverse divisions. These outer cells are nearly uniform, and show not even the beginning of any differentiation into stomium or annulus; consequently, there is nothing to indicate the ancestry of *Marsilia*, so far as this might be determined by annulus characters.

The microsporangia, which appeared as sister cells of the megasporangium, are at this time found below the megasporangium. While the megasporangia were developing sporogenous tissue, the apical cell of the microsporangia was cutting off segments to form

an elongated stalk. When the megasporangium reaches the sixteen-spore mother cell stage, the microsporangia cease to add any more segments to the stalk; a transverse wall through the apical cell of



FIGS. 1–4.—Fig. 1, megasporangium with single archesporial cell (*a*); *m*, microsporangia; fig. 2, primary sporogenous cell (*s*); fig. 3, quadrant stage; tapetum (*t*); fig. 4, octant stage.

the microsporangium cuts off a cap cell, and a central tetahedral archesporial cell is established.

The megaspore mother cells are so elongated that in longitudinal or transverse sections the group of sixteen cells is nearly twice as

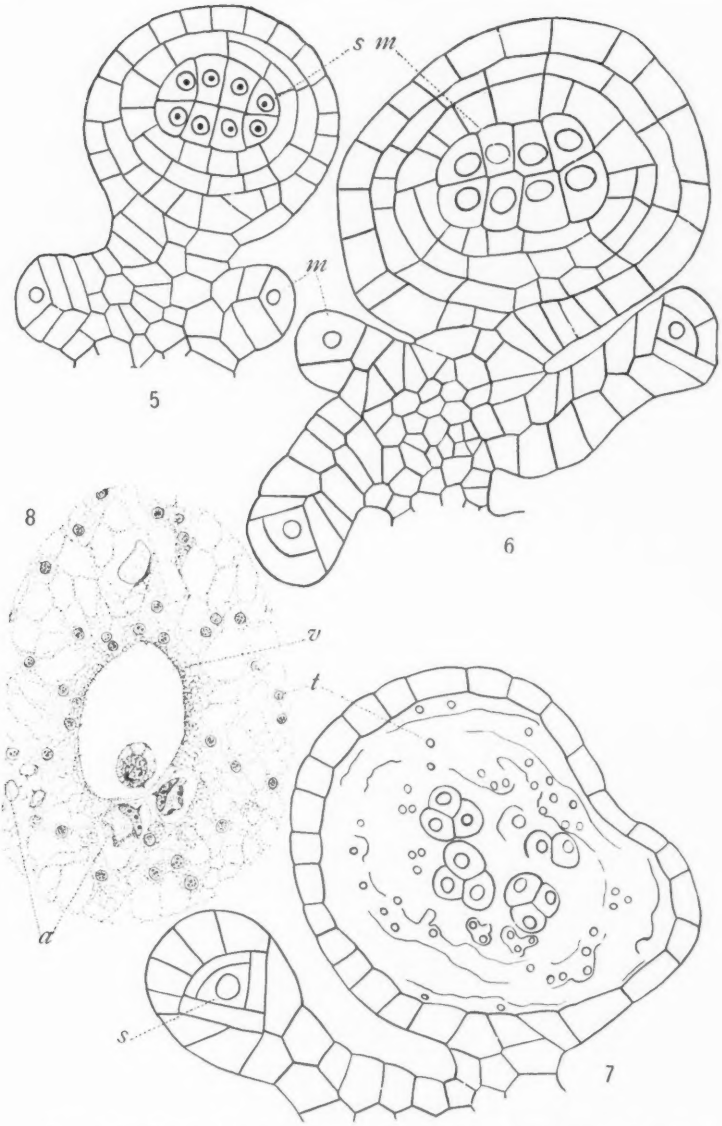
long as broad. They are easily distinguished from the tapetum and the epidermal cells by their denser finely granular cytoplasm, and by the fact that the nucleus soon becomes very large. The nuclei of the sporogenous cells are larger than those of the tapetum, and the nuclei of the tapetum are larger than those of the epidermis.

A number of mitotic figures in the nuclei of the sporangia showed the chromosomes in synapsis, diakinesis, anaphase, metaphase, and telophase. An examination of the nucleus in synapsis shows a clear space at one side. The chromatin is in a somewhat contracted mass at the other side, and the outline of the individual chromosomes is just discernible. The nucleolus may be closely associated with the chromatin mass or it may be at some distance. The nuclear membrane is quite distinct.

A pair of large chromosomes is observed in an early diakinesis stage. The sixteen pairs of chromosomes are attached to each other by threads. A little later the pairs become more clearly differentiated and the single large pair stands out more distinctly. The continued appearance of this large pair of chromosomes suggests that they may be sex chromosomes.

The *x* or haploid generation of *Marsilia* is very short, since there are only a few divisions from the megaspore up to the egg ready for fertilization; and there are also only a few divisions from the microspores to the production of sperms. At fertilization a monoecious *2x* generation is established, bearing both megasporangia and microsporangia in the same sporocarp, so that the segregation of the sexes must have taken place in some somatic mitosis at an early stage in the development of sporangia. Since the megasporangia and microsporangia are derived from sister cells, sex differentiation should occur in the formation of these cells. The fact that in the megasporangium the spore mother cell showed a large pair of chromosomes may indicate that segregation has occurred previously. As soon as more material is available, the chromosomes of the microspore mother cell will be examined and compared with the megaspore mother cells and with the somatic cell from which they are derived. Should these three differ in chromatin content, this will be conclusive evidence that the segregation of sexes occurred as stated.

With the reduction divisions in the sixteen megaspore mother



FIGS. 5–8.—Fig. 5, sixteen megaspore mother cells; microsporangia (*m*); fig. 6, same slightly older; fig. 7, megasporangium with tetrads; tapetal plasmodium (*t*); microsporangium with archesporial cell (*s*); fig. 8, functioning megaspore; vacuole (*v*); tapetal nuclei (*t*); abortive spores (*a*).

cells, sixty-four spores are formed. Each tetrad of spores hangs together by strands, and all are surrounded by protoplasm and nuclei of the tapetum, cell walls of which have been dissolved (fig. 7). The microsporangium at this time shows that the first tapetal cells have been cut off and the epidermal cells have divided transversely.

Only one of the sixty-four megaspores ever reaches maturity. The centrally placed spore which gains ascendency is surrounded by a mass of vacuolated cytoplasm, in which the disintegrating tapetal nuclei as well as the abortive spores may be found. This mass is gradually assimilated by the maturing megaspore (fig. 8). Two megasporangia are sometimes found developing simultaneously in a single sorus (fig. 9). At this time the microsporangia have reached the sixteen-spore mother cell stage. Very soon each spore mother cell forms a tetrad of microspores, the tapetal walls are dissolved, and the nuclei and protoplasm of these cells surround the microspores.

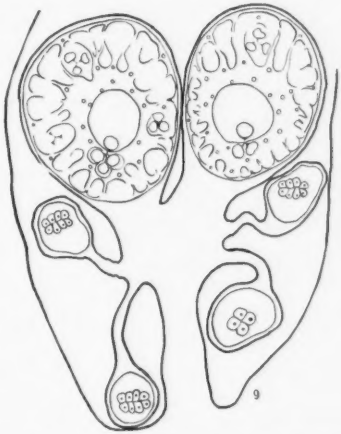


FIG. 9.—Diagrammatic section of two megasporangia; microspore mother cells.

Finally, the megasporangia and microsporangia reach their full development at the same time; in the former only one megaspore maturing, while in the latter sixty-four microspores mature. A single layer of epidermal cells is all that remains of the sporangia, and this layer, even in the latest stages, shows no indication of an annulus or any special structure for dehiscence.

Comparison with other heterosporous forms

It is interesting to compare *Marsilia* with the other heterosporous Pteridophytes, and to note where the differentiation of sporangia occurs in each.

In *Selaginella* (4) the sporangia develop nearly alike up to the

spore mother cell stage, but, even before sporogenous divisions cease, a slight elongation distinguishes the microsporangia, while the megasporangia can be identified by a more nearly spherical shape. In the microsporangia all the mother cells function and numerous microspores are formed, while in most species in the megasporangia all but one of the spore mother cells abort and supply nutrition to the four functioning megaspores.

In *Isoetes* (**7**) the sporangia develop alike until they reach the 15,000–20,000 cell stage. Then, in those sporangia which are to form microspores, some of the sporogenous cells are diverted and form trabeculae which extend across the sporangium, while the remainder function in forming the microspores, varying in number from 150,000 to 300,000. In those which are to form megaspores, some of the sporogenous tissue forms trabeculae, which, however, are more massive; the remainder function in the production of the 150–300 megaspores.

In the sporocarp of *Azolla* (**5**) the megasporangium develops first, followed soon by the microsporangium, after which comes a period of simultaneous growth. Observation shows that if the apical sporangium gains the ascendency, it becomes a megasporangium, and lateral sporangia abort. Within the functioning megasporangium 31 of the 32 megaspores abort, leaving one functioning megaspore. On the other hand, if the apical sporangium aborts the lateral sporangia become microsporangia, and their 30 spores become microspores.

In *Salvinia* (**9**) the microsporangia and megasporangia develop in different sporocarps. Several megasporangia are produced in a single sporocarp, each sporangium producing one megaspore. In the microsporocarp the numerous sporangia mature all their spores.

In *Pilularia* (**3**) the first sporangia formed, near the base of the sorus, produce megaspores, while the upper and younger ones produce microspores. As in *Marsilia*, the sporangia are all produced in the same sporocarp. While this does not show exactly where differentiation of sex in the water ferns occurs, it at least indicates, within rather narrow limits, where any structural differentiation is to be sought.

Summary

1. The microsporangia and megasporangia of *Marsilia* are derived from sister cells.

2. The stalk of the microsporangium is very much longer than that of the megasporangium.

3. In the spore mother cell stage the microsporangia are elongated and oval, while the megasporangia are somewhat flattened.

4. The sporangia differ in their time of development. In the megasporangium there is a gradual continued development of the sporogenous tissue; in the microsporangia there is delay in the development of the sporogenous tissue, while the apical cell cuts off segments to form an elongated stalk.

5. The megaspore mother cell in diakinesis shows a pair of large chromosomes which may be sex chromosomes.

6. Since *Marsilia* is a monoecious plant, sex differentiation must have occurred in some somatic mitosis.

7. When the single functioning megaspore has gained ascendency, the sporogenous tissue of the microsporangia resumes growth and develops very rapidly.

8. The protoplasmic strands between the tetrads of megaspores are much stronger and more persistent than in the microspores.

9. The functioning megaspore is surrounded by a mass of vacuolated cytoplasm, in which the tapetal nuclei as well as the aborted spores may be found.

10. In each of the six genera of heterosporous Pteridophytes the difference in the sporangia becomes visible at a different stage. If the chromosomes were examined it would probably be found that the differentiation of sex had occurred in an earlier somatic cell.

Grateful acknowledgment is made to Professor CHARLES J. CHAMBERLAIN for criticism and suggestions.

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SYMBIOSIS IN A DECIDUOUS FOREST. II

W. B. McDOUGALL

(WITH ONE FIGURE)

The first paper of this series[1] was devoted to an ecological description of University Woods, in Champaign County, Illinois, and to a general discussion of social disjunctive symbiosis as exhibited therein. It is the purpose of the present installment to discuss briefly some instances of nutritive disjunctive symbiosis and their effect on the vegetation in this same woods. It should be understood that this is not intended as an exhaustive treatment of the subject, which would require many years of observation and study. The purpose is rather to present the relatively few examples that have been studied during the past few years, not only as a contribution to our knowledge of symbiosis, but also to illustrate what kinds of phenomena should be included under nutritive disjunctive symbiosis.

Antagonistic nutritive disjunctive symbiosis

The phenomena that are classed under this type of symbiosis are those in which two or more unlike organisms are living together (not in actual contact, at least not all of the time) and one or more (but not all) kinds of organisms deriving food from the others. All ordinary cases of the interrelations of herbivorous animals and plants, therefore, belong in this category.

Before the University of Illinois obtained possession of the University Woods some seven years ago, it was much grazed by cattle. This had the effect of practically eliminating some species of herbaceous plants and of greatly reducing the numbers of others, while reproduction of the trees was for the time being almost completely stopped. The effects of this overgrazing are still very evident. Since the woods was acquired by the University there has been no grazing, and reproduction of the trees is now progressing satisfactorily. Certain herbaceous plants which were unpalatable to the

[1] BOT. GAZ. 73:200–212. 1922.

cattle survived the period of grazing in such numbers that they were able to take advantage of the weakened condition of other species and spread rapidly. A notable example of this is *Laportea canadensis*. Fig. 1 shows the present distribution of this plant. The species is undoubtedly much more abundant than it would be if the woods had never been grazed. Another plant which has likewise become very abundant, especially in those parts of the woods not occupied by the nettle, is *Eupatorium urticaefolium*.

Other herbivorous mammals have not been important in this woods. Rabbits feed upon various kinds of plants without doing much damage. Squirrels, mice, and perhaps a few other rodents feed upon nuts and other fruits, but they have never been abundant enough to become very important symbionts.

Instances of antagonistic, nutritive, disjunctive symbiosis between insects and plants are numerous. The majority of these, however, do not have any very marked effect on the life of the plants, although occasionally some of them do. Perhaps the most effective case observed is that of some unidentified lepidopterous larvae on the wood nettle.[1] These feed upon the leaves, and are often so abundant during late summer and early autumn that it is scarcely possible to find a single intact leaf. Such a condition is rather serious for the plant, of course, although it is unlikely that the plants are ever actually killed by the larvae. Several kinds of lepidopterous larvae were also found feeding on the leaves of *Benzoin melissaefolium*.

Lace bugs were found rather commonly on the under side of the leaves of *Asimina triloba*, where they feed on the plant juices. The tarnished plant bug, which feeds entirely on plant juices, was found frequently on several species of *Aster* and *Solidago*. Aphids, often attended by ants, were found on various kinds of plants, especially members of the composite family. These are only a few of the cases of symbiosis between insects and plants that might be found in such a forest, but they all represent interrelations entirely comparable with that of the well known leaf-cutting ants and the fungi which they cultivate, and therefore should be classified under the same type of symbiosis, namely antagonistic, nutritive, disjunctive.

[1] The observations on symbiosis between insects and plants recorded here were made by Mr. CURTIS BENTON.

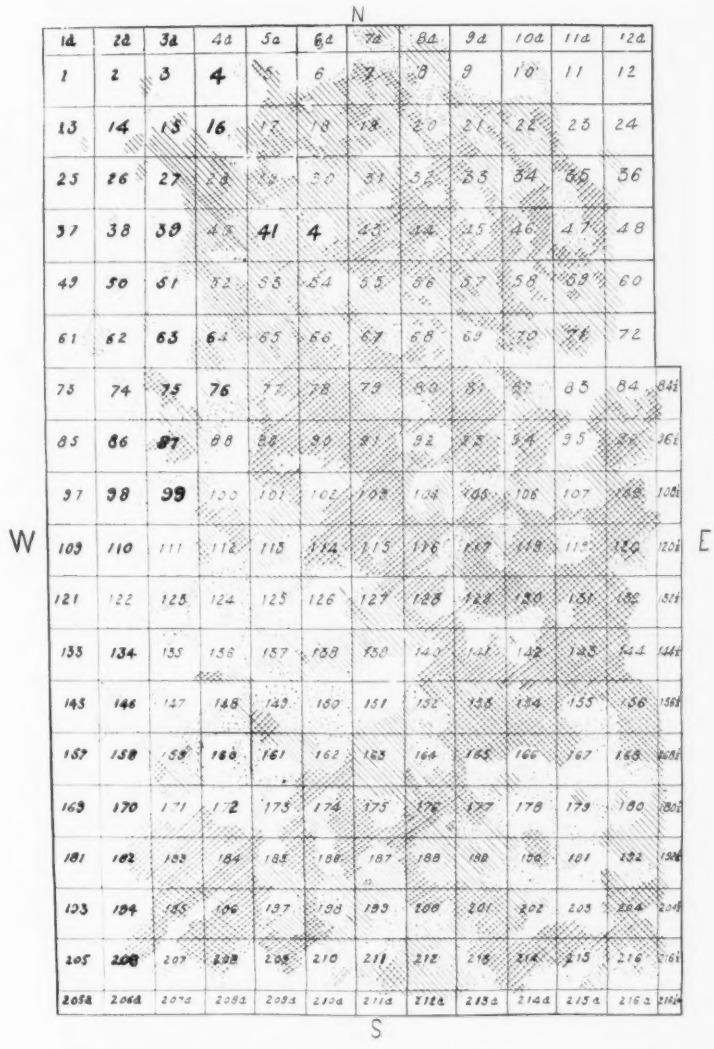

FIG. 1.—Present distribution of *Laportea canadensis* in University Woods: double diagonals, dense growth; diagonals, medium density; dots, scattered plants; chart prepared by P. E. GROVE.

The most conspicuous example of this type of symbiosis in the University Woods, and the one that produces the greatest effect on the vegetation, is that between the domestic fowls and the plants. There are two farmhouses across the road from the woods on the west. One of these is opposite quadrat no. 73 (fig. 1) and the other opposite quadrat no. 157. A flock of chickens is maintained at each of these farms. These enter the woods daily and occasionally go as far as half way across the woods. Usually they do not go so far, however, and the effects of their presence is most noticeable in the first two or three rows of quadrats. The chickens feed upon the plants directly to a certain extent, and they also do considerable damage by scratching the soil in search of insects and other food. The greater part of the food obtained by the chickens is probably not obtained directly from the plants, but from the point of view of the chickens, their relationship with the plant community concerns their food supply, and therefore the symbiotic relation is a nutritive one. On the other hand, the plants do not get any food from the chickens and probably are not benefited in any other way by their presence. Therefore the relationship is an antagonistic one.

The method used for estimating the effect of the presence of the chickens on the vegetation was as follows. It will be noticed from fig. 1 that the woods is divided into 216 full quadrats, with half quadrats along each end and part way along the east side. Each of the full quadrats is 100 feet square, and each half quadrat is 50 by 100 feet. At one time the corners of all of these quadrats were marked by stakes, but these have mostly disappeared. It was decided to get a comparison between the west and east sides of the woods by counting the total number of plants of all species in a series of meter quadrats along each side 100 feet from the fence. It was further decided to locate these meter quadrats at every 100 feet. Starting at the southwest corner of the woods, therefore, the first quadrat was located by pacing 33 paces east and 33 paces north to the middle of the north and south boundary line between quadrats 205 and 206. Then using a compass to keep on the line, the next quadrat was located by walking 33 paces due north to the middle of the line between quadrats 193 and 149, and so on until the series had been completed. On the east side of the woods the same method was used, but in the southern two-thirds the meter quadrats

fell in the middle of the large quadrats of the first row of full quadrats, instead of on the line between the first and second rows, as on the west side and in the northern third of the east side, this variation being due to the jog in the fence on this side of the woods.

A folding wooden frame one meter square which was easily carried about was used, so that when the position of a quadrat had been located it was only necessary to drop the frame and proceed at once to count the plants within it.

TABLE I

West side		East side	
Quadrat	No. of plants	Quadrat	No. of plants
A	25	a	70
B	32	b	41
C	12	c	21
D	15	d	49
E	9	e	54
F	3	f	59
G	34	g	56
H	6	h	54
I	19	i	62
J	80	j	30
K	33	k	90
L	28	l	71
M	21	m	41
N	12	n	42
O	5	o	45
P	16	p	59
Q	33	q	44
R	25	r	45
Average	22.7		51.8

The plants in the eighteen quadrats thus located on the west side of the woods were counted on June 5, 1923, and those on the east side were counted two days later, on June 7. A second counting was made in the quadrats on the west side on August 27 and on the east side on August 30. In order to avoid confusing the meter quadrats with the one hundred feet quadrats, the small ones were designated by letters instead of numerals. Beginning at the south end of the woods they were lettered from *A* to *R* on the west side of the woods, and from *a* to *r* on the east side. The results of the first counts made, those of June 5 and June 7, are shown in table I.

It is seen from table I that the average number of plants per meter quadrat on the west side of the woods where the chickens work is less than half that on the east side. The total number of species represented in the 18 quadrats on the west side of the woods was 31, while in the 18 quadrats on the east side there were only 28 species represented. This difference is to be explained by the fact that the activities of the chickens in destroying the native plants made possible the entrance of a number of ruderal species which were not represented on the east side at all. Nineteen species were common to the quadrats on the two sides of the woods.

The area of greatest activity of the chickens, however, is confined to about 500 feet of north and south extent for each flock. Therefore, if we disregard quadrats *A*, *B*, *C*, and *D* at the south, *J* and *K* in the middle, and *Q* and *R* at the north, we have left the ten quadrats that show the effect of the chickens to the greatest extent. The ten quadrats on the east that can best be compared with ten on the west are those at the south end, *i* to *r* inclusive, since they are most like the west side of the woods as regards dominant species; the south end of the east side and practically all of west side being in the maple consociation, while the north end of the east side is in the elm consociation.

Comparing these selected quadrats, we find in the ten on the west side a total of 153 plants, or an average of 15.3 per quadrat, and in the ten on the east side a total of 496 plants, or 49.6 per quadrat. The total number of species represented in the ten quadrats on the west side was 21, while the total number on the east was 18. Twelve species were common to the two sets of quadrats.

The results of the counts made in August are shown in table II. The totals are lower than in June, owing to the fact that the vernal plants had for the most part disappeared and their places had been taken by the larger plants of the aestival season, but in general the results are similar to those obtained in June.

The total number of species represented in the 18 quadrats on the west side in August was 27 and on the east side 21, while eleven species were common to the two sets of quadrats.

By using only the ten selected quadrats on each side of the woods, as was done for the June counts, we find the average number

of plants per quadrat on the west side to be 12.2 and on the east side 28.4. The total number of species represented in the ten quadrats on the west side was 16 and in those on the east 11, while only four species were common to the two sets of quadrats.

The conclusion may be drawn from these data that the presence of the chickens in the woods tends to reduce greatly the number of individual plants but to increase the number of species. This

TABLE II

West side		East side	
Quadrat	No. of plants	Quadrat	No. of plants
A	39	a	21
B	35	b	33
C	20	c	39
D	14	d	14
E	16	e	26
F	3	f	25
G	11	g	26
H	30	h	35
I	14	i	25
J	13	j	40
K	26	k	32
L	14	l	34
M	10	m	31
N	10	n	27
O	5	o	18
P	9	p	27
Q	16	q	26
R	19	r	24
Average	16.8		28

latter effect, as just stated, is due to the introduction of weedy plants which are out of place in the forest and could not possibly compete with the forest plants under normal conditions. The presence of the chickens is therefore distinctly detrimental to the forest vegetation; it is a clear case of antagonistic, nutritive, disjunctive symbiosis.

Reciprocal nutritive disjunctive symbiosis

In this type of disjunctive symbiosis one or more, but not necessarily all, of the organisms concerned get food from one or more of the others, but those that lose food and get none in return are never-

theless benefited by the relationship in some other way, so that the symbiosis is reciprocal so far as actual benefit derived from it is concerned. The outstanding examples are those concerned with pollination by means of animals, especially insects, and with dissemination through the aid of animals, especially birds and ants. Since my observations on this type of symbiosis have not brought out any new facts, a brief statement of the extent to which it occurs in this woods will suffice.

Of the 132 species of herbaceous plants occurring in the woods, 101, or approximately 76 per cent, are pollinated by insects, while the insects are getting food from the flowers. Of the 31 species of trees, only 9, or approximately 29 per cent, are insect pollinated; but all of the twelve species of shrubs and all of the six species of lianas have this symbiotic relation with insects. It will be seen that the small amount of space I am giving to this type of symbiosis is not in proportion to its importance to the plants.

Dissemination through the aid of animals is not so widespread as is pollination, but it is by no means unimportant. There are, of course, a number of plants with burlike fruits which are disseminated by animals, but those cases do not represent the type of symbiosis now under consideration, because there is no food relation. Birds are by far the most important agents which bring about dissemination through their activities in connection with their food supply. Thirteen herbaceous plants, or about 10 per cent of the total number, are disseminated to a certain extent by birds. Six species of trees, or 19 per cent of the total number, and all of the twelve species of shrubs and six species of lianas exhibit this type of symbiosis.

Dissemination by insects is not so common, but ants feed upon the large crests of the seeds of *Sanguinaria canadensis* and upon the fleshy raphes of the seeds of *Asarum canadense*, and frequently carry the seeds from place to place, thus aiding in scattering them. They probably also have a similar symbiotic relation with *Floerkia proserpinacoides*, feeding upon the rough, fleshy outer portions of the akenes, although further observations are needed upon this point.

UNIVERSITY OF ILLINOIS
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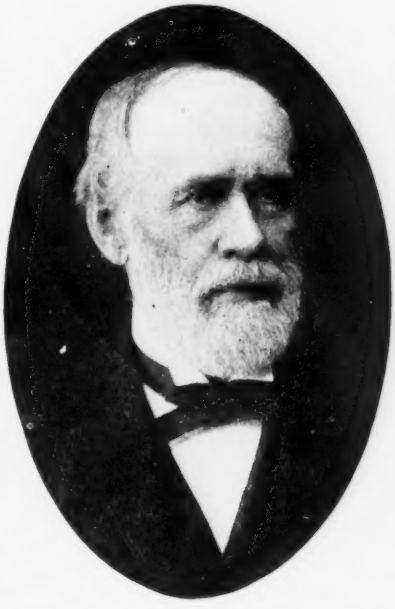
BRIEFER ARTICLES

WILLIAM JAMES BEAL

(WITH PORTRAIT)

With the death of Dr. BEAL, May 12, 1924, there passed away the last of those leaders of botany who, in the latter half of last century, were changing the course of botanical thought from the idea of systematic botany as the end and aim of all botany to the study of all kinds of plants from all standpoints.



Dr. BEAL was born at Adrian, Michigan, March 11, 1833, of Quaker stock. His parents were pioneers in Michigan, which was then, for the greater part, still wilderness. Taking advantage of the meager opportunities for schooling, he made his way through the district school and seminary and entered the University of Michigan, from which he graduated in 1859. Most of his education was obtained at the cost of hard work, for it was necessary for him to earn his way through the academy by the then usual method of teaching school during the winter. After graduation, he became teacher of Natural Science at the Friends' Academy, and later at Howland Institute at Union Springs, New York. This position he held, except for absences for the purpose of further study, from 1859 to 1868. During vacations and other periods he found opportunity to study at Harvard, coming under the influence of LOUIS AGASSIZ and ASA GRAY, the former probably making the greater impression upon him, as shown in

his teaching methods later. In 1865 he received the degree of B.S. from Harvard.

In 1868 the original University of Chicago called him to be Professor of Botany, a position which he held for two years. Upon the call of Professor PRENTISS from the Michigan Agricultural College to Cornell University, Dr. BEAL was made lecturer in botany for part of the year 1870 at Michigan. In 1871 he became a full member of the faculty as Professor of Botany and Horticulture. In 1882 the Department of Horticulture was established, and he then became Professor of Botany and Forestry. When a separate Department of Forestry was formed in 1903, Dr. BEAL was relieved of the forestry work and became Professor of Botany alone. In 1910, at the completion of his fortieth year of connection with the Michigan Agricultural College, he retired as Professor Emeritus and went to Amherst, Massachusetts, where he resided at the home of his daughter, Mrs. RAY STANNARD BAKER, until his death.

Dr. BEAL was always a man of abstemious habits, to which, coupled with his habitual practice of exercising, he ascribed his freedom from illness up to about the very end of his life. When far past his eightieth year he ran a few hundred feet every day or sawed a set number of sticks of wood. He outlived most of his University of Michigan classmates, and at the time of his death was the oldest graduate of that University, and the oldest male resident of Amherst, Massachusetts.

Interested in the diffusion of scientific knowledge, Dr. BEAL associated himself with various scientific societies. He was one of the organizers and the first president of the Society for the Promotion of Agricultural Science, and charter member and first president of the Michigan Academy of Science. He was Director of the State Forestry Commission from 1888 to 1892, Fellow of the American Association for the Advancement of Science (chairman of the Section of Biology in 1895), Secretary of the American Pomological Society from 1881 to 1885, President of the Michigan State Teachers' Association, member of the Botanical Society of America, and of a number of other societies. In all these he took an active part until about the time of his retirement in 1910.

As a writer, Dr. BEAL's most extensive botanical contribution was the two volume work *The grasses of North America*, published in 1887 and 1896. He published a small booklet in 1879 entitled *The new botany*, and one in 1898 on *Seed dispersal*. His greatest literary activity, however, was in articles for botanical periodicals and for horticultural and agricultural papers. Particularly numerous were the papers of the last class, and it was by means of these that Dr. BEAL became so widely known among the citizens of Michigan and adjoining states.

Probably it was as a teacher that Dr. BEAL had his widest influence. He came to the teaching profession with a breadth of experience and training unusual in those days. As a boy and young man he knew the practical side of agriculture and horticulture from personal experience. His university course was of the classical-literary type, the only kind available then, apart from the professional courses leading to law, medicine, or the ministry. At Harvard, under GRAY and AGASSIZ, he studied botany and zoology, subjects which had always been of great interest to him. Under AGASSIZ he first encountered the laboratory as a method of instruction in biological science, for GRAY's undergraduate courses at that time were still given only as recitations from a text-book, without requiring the students to see the plants mentioned. This made a lasting impression on Dr. BEAL, so that when he came to Michigan Agricultural College he was not satisfied to teach these subjects by the text-book and recitation method. It was not long before he managed to set aside a small space for a botanical laboratory, and planned for a laboratory building. An interesting fact is that GOODALE of Harvard, BESSEY of Iowa, BURRILL in Illinois, and BEAL in Michigan, independently of one another, all adopted the laboratory idea for teaching undergraduate botany at about the same time, in the early seventies. In 1879 Dr. BEAL succeeded in obtaining an appropriation to build a botanical laboratory, a two-story wooden building, one of the first buildings in this country to be devoted to botany alone. This was occupied in the spring of 1880, and was destroyed by fire in 1890, but the general herbarium, apparatus, and books were saved.

In addition to his manifold duties as Professor of Botany and Horticulture, with no assistance in teaching, Dr. BEAL started a botanical garden in 1877. This is, therefore, one of the older botanical gardens of the country, and at the close of Dr. BEAL's service in 1910 it contained over 2000 species.

In 1879 Dr. BEAL began his well known experiment with buried seeds. These seeds were mixed with soil and placed in uncorked bottles, buried mouth downward in the ground at a depth of 16–24 inches. Every five years one bottle was dug up and the seeds germinated, and since Dr. BEAL's retirement this has been continued. At the fortieth year eleven kinds of seeds still showed germination out of the twenty kinds placed in the bottle originally.

Dr. BEAL held ideas that were far in advance of the time. In the mid-seventies he called attention repeatedly to the folly of the destruction of the forests, and advocated the treatment of forests more in line with what is now considered to be sane forest conservation. He was ridiculed greatly for his fears for the "inexhaustible forests of Michigan," but lived to see

the day when the state began the replanting of cut-over land. As a recognition of his interest in forestry, he was made Director of the State Forestry Commission from 1888 to 1892.

The final botanical publications of Dr. BEAL were two bulletins on *Seeds of Michigan weeds* and *Michigan weeds*. For over thirty years he had studied the problem of agricultural seeds and their impurities and poor quality, contending for cleaner, better seed. In 1877 he began testing seeds for purity and viability long before the first seed laboratory was established in this country. In 1910 his bulletin on *Seeds of Michigan weeds* showed that he had not yet lost interest in this important subject. It would be impossible to list the thousand or more articles he contributed to various agricultural and horticultural journals.

After his retirement, Dr. BEAL moved to Amherst, Massachusetts, giving up all botanical work. His large collection of botanical separates he gave to the college, and spent a year or two writing a history of Michigan Agricultural College. Although ceasing botanical work, he did not lose his interest in plants, and almost up to the time of his death he worked in his garden, set out shrubbery, and dispensed information of practical value on these subjects to his friends and neighbors.—ERNST A. BESSEY.

EFFECT OF LIGHT ON AVAILABILITY OF IRON TO WHEAT PLANTS IN WATER CULTURES

(WITH ONE FIGURE)

How different climatic complexes may markedly affect the growth efficiencies of aqueous culture media, can be very nicely demonstrated in experiments with nutrient solutions devoid of iron. It appears that deficiency or absence of this element in the media has a much more deleterious effect on plants growing in bright sunlight than it has on those growing under conditions more or less shaded.

So markedly does light affect the availability of or the requirement of the plant for iron, that demonstrations for the classroom on the effect of this element on the greening of plants often fail to give the desired results because of certain photogenic conditions. Fig. 1 shows some of the effects of two different light intensities on the growth of wheat in nutrient solutions devoid of iron; the chief points of difference in the plants being that those exposed to bright sunlight were much more etiolated, showed marked abbreviation in length of leaves and stalks, and possessed more incipient tillers than did those plants exposed to less sunlight.

The features of the plants exposed to the high light intensity apparently are the result of conditions that produced a higher rate of operation of certain growth processes than prevailed with the plants grown under a lower light intensity. Due to these differences in rates of processes, the requirement of the plant for iron may vary. It appears that the need for this material is much greater per unit of growth of plant exposed to high light intensities than to low ones.

FIG. 1.—1, wheat plants grown in solutions devoid of iron and exposed to bright sunlight; 2, wheat plants grown in similar solution devoid of iron, but exposed to less light.

The marked etiolation of the plants exposed to bright sunlight has been shown to be related to certain effects of light on chlorophyl. Apparently both the amount of growth, and the stage of growth that accrues from the operation of specific rates of various processes, can be affected by this agency. Although the plants exposed to bright sunlight were much smaller than those not so exposed, the former were more matured than the latter; that is, exposure of the plants to conditions of relatively high light intensity without iron in the media resulted in more matured but stunted plants, whereas with iron in the media under the same light conditions the plants would have been larger. Thus conditions, which in the presence of iron in the culture media are decidedly beneficial to growth, become conditions relatively harmful in the absence of iron. These results, therefore, indicate that there can be no fixed minimum amount of

iron for a unit yield of product, and as this principle applies to iron, so may it possibly apply to all other elements required for the growth of plants. The amount of material needed by plants for a unit yield of product would be determined by rates of other processes that are part of the complex-growth.

While many of the synthetic products of plant origin have a definite chemical composition and structure, and therefore involve stoichiometric relations of their component parts, nevertheless, as the composition of plants is, in a sense, a mixture of various chemical compounds, it follows that any change in the proportion of the components of this mixture must also affect the amount of any given element required per unit growth of plant.—W. F. GERICKE, *Laboratory of Plant Nutrition, University of California.*

CURRENT LITERATURE

BOOK REVIEWS

Textbooks of general botany

HOLMAN and ROBBINS,[1] of the University of California, have published a textbook of general botany, which contains the material presented in two courses, one for agricultural students at the Branch of the Agricultural College at Davis, and one for both agricultural and liberal arts students at Berkeley. Part I begins with the cell, followed by chapters on the plant as a whole, stem, root, leaf, flower, fruit, seed, and seed germination. Part II presents the four great groups of the plant kingdom, closing with a chapter on evolution and heredity.

Every teacher with any initiative has his own method of presenting the facts of his subject, and in consequence textbooks of botany are multiplying rapidly. It is very interesting to discover in this way the methods of different teachers. The present volume is written in clear style, including much detail, and is amply illustrated. Among the illustrations used there are selections from a wide range of authors. Naturally, the presentation of the material has been adjusted to the needs of the particular groups of students involved, and has been tried with the student groups before publication.

A group of botanists at the University of Wisconsin has co-operated in the publication of a textbook of general botany.[2] It is based upon the conviction that botany should be presented as a unit, and is an outgrowth of the experience of the writers in the teaching of elementary botany. Especially have they emphasized the fact that structure and function should be intimately associated. The six authors are not differentiated in the presentation, but it is a synthesis of their training and teaching experience. The result is certainly interesting and very suggestive, as is also the sequence of presentation.

The first twelve chapters deal with the general structures and functions of plants, beginning with the cell, and proceeding through roots, stems, buds, and leaves to water relations, food manufacture, plastids and pigments, food utilization, stimulus and response, and embryogeny. The following eighteen chapters deal with the great groups, beginning with algae and closing with seeds and fruits. A chapter between mosses and ferns is devoted to an account of reduction of the number of chromosomes. The closing chapters deal with inheritance and variation, evolution, geographic distribution, and economic significance of

[1] HOLMAN, R. M., and ROBBINS, W. W., A Textbook of general botany. 8vo. pp. vii+590. *figs. 374.* New York: John Wiley & Sons. 1924. $4.

[2] SMITH, G. M., OVERTON, J. B., GILBERT, E. M., DENNISTON, R. H., BRYAN, G. S., and ALLEN, C. E., A textbook of general botany. 8vo. pp. x+409. *figs. 321.* New York: Macmillan Co. 1924.

plants, the last under the following subtitles: crop plants, plants used in medicine, forestry and forest products, weeds, and plant diseases.

The book is a very suggestive one for teachers, and is a valuable contribution to the problem of presentation.—J. M. C.

Genetics

The recently published text on genetics by JONES[3] is distinctly up-to-date, comprehensive, and practical. The thorough way in which recent literature is covered makes it extremely valuable as a reference book; and the practical flavor of the many impressive examples is a feature which should be of great advantage in agricultural schools. These very features might render it somewhat cumbersome as an elementary text, where the teacher plans to lay a foundation of pure theory at the beginning; but the author has in mind the agricultural student, and makes theory subservient to practice.

Chapter I sketches the rôle of genetics in agriculture, depicting its possibilities and its limitations. Chapter II discusses the influence of environment, emphasizes the necessity of distinguishing effects of environment from those of heredity, and treats in a practical way the question of inheritance of acquired characters. Chapter III explains why bisexual reproduction brings greater variation, and points out the advantages which result. Chapter IV presents, in a rather concentrated form, monohybrid and polyhybrid breeding results, together with examples of all types of factor interactions. Chapter V discusses the chromosome mechanism, linkage and crossing over, multiple allelomorphs, lethals, semi-lethals, and balanced lethals. Chapter VI takes up sex and sex-linkage, with the conclusion that sex control is hopeless. Chapter VII classifies variations from several points of view. "All mutations may ultimately be understood as the results of an orderly process." Mutations are so rare as to be relatively unimportant in the practical business of plant and animal improvement, as compared with the possibilities of recombining existing hereditary units through hybridization. Chapter VIII puts practical statistical methods in a readily available form. Chapter IX provides a thorough discussion of the efficacy of selection within populations of various types. Chapter X describes, by means of numerous examples, the part that has been played by hybridization in the production of cultivated plants and domesticated animals. Chapters XI and XII cover the same field as "Inbreeding and outbreeding."[4] Some more recent evidence is added, but the previous interpretation remains unmodified. Nowhere has the reviewer encountered a more comprehensive treatment of the subject of sterility than appears in chapter XIII. All types of sterility are presented, including a thorough discussion of species and genus hybrids. Chapters XIV and XV furnish a very fitting conclusion; they contain specific recommendations,

[3] JONES, D. F., Genetics in plant and animal improvement. 8vo. pp. viii+568. *figs. 229*. New York: John Wiley & Sons. 1924. $5.

[4] EAST, E. M., and JONES, D. F., Inbreedir g and outbreeding. Philadelphia. 1919.

as to technique and general breeding program, for the improvement of all our more important agricultural types.

The text is illustrated by 229 figures and numerous tables. It carries a glossary of 114 terms and a bibliography of 312 titles.—M. C. COULTER.

Desert conditions

As plants constitute the most important part of animal environment, it is not surprising that a recent volume dedicated to desert animals[5] should have not less than half its space devoted to plants and to conditions that limit their development. In discussing the factor of water, emphasis is placed on the fact that not only is precipitation decidedly limited, but it is usually liable to very great variation from monthly and annual averages. Thus average rainfall is not an accurate indicator of desert conditions, because a large proportion comes as non-available moisture, or because much of it falls in the cold season, and because from year to year the precipitation deviates widely from the normal.

In considering desert temperatures, attention is directed to the wide yearly and daily range. Instances of variations, such as from $-0.5°$ to $37.2°$ C., and from $3°$ to $28°$ C. within 24 hours are cited. Surface soil exposed to the sun may show temperatures as much as $20°$ C. above that in the shade, with such maxima as $60°$ C. in Palestine, $84°$ C. on the Loango Coast, and $78°$ C. in the Sahara. Other climatic factors are well summarized, and this summary, considered along with the condition of soil and water courses, is so presented as to form a good picture of desert conditions.

In addition to the food relations of plants and animals, considerable space is devoted to the shelter afforded animals by certain plants. Thus two woodpeckers are found most abundantly within the range of the giant cactus, *Cereus giganteus*, in whose stems they roost. The small elf owl is found exclusively in deserts where this cactus grows, using the abandoned woodpeckers' holes for its nests. Several other birds are somewhat less dependent upon the cactus and its woodpecker holes, but have an extended range where these occur.

The chapters on the physical environment of desert animals and on their colors are interesting, and go to make the book an important one in bringing graphically before the reader a picture of desert conditions. Well chosen illustrations add to the interest of the volume.—GEO. D. FULLER.

Transpiration and sap ascent

Plant physiologists, to whom DIXON's monograph has long been the classic work, will welcome the small volume[6] recently published which brings up-to-date the work in this field. The first of these three lectures, delivered before the University of London, treats of the criticisms (adverse and favorable) which have

[5] BUXTON, P. A., Animal life in deserts. A study in the fauna in relation to the environment. London. 8vo. pp. vii+176. *figs.* 43. 1923. Edward Arnold & Co. 10/6.

[6] DIXON, H. H., The transpiration stream. London: Univ. of London Press. pp. 80. 1924.

been offered of the theory of a continuous column of water in the tracheae. The researches of HOLLE, BODE, URSPRUNG, RENNER, and NORDHAUSEN are reviewed, and the conclusion is reached that the cohesion theory stands today more firmly supported by experimental evidence than ever before.

In the second lecture DIXON discusses the work of BOSE, and records his inability to obtain the same results with the same methods. The more important work of URSPRUNG and BLUM on the living cells of the vascular system is also considered quite carefully, and it is concluded that as yet there is no need of assuming the intervention of any vital cell processes in the ascent of sap.

The third lecture constitutes a valuable summary of the very considerable experimentation of the lecturer toward the solution of the problem of the movement of organic substances in the plant. Naturally only such other researches are considered as have a direct bearing on the author's own theory of the transport of organic matter by the transpiration stream, but they are marshalled in formidable array. In the preface, however, a moderate position is assumed, and the outstanding need presented is not the acceptance of this new view, but renewed experimentation to ascertain the truth.

The bibliography of over sixty titles serves to make this truly a supplement which bridges over the decade since the publication of the monograph, and brings together all the literature on transpiration and sap ascent up to the past year.—H. S. WOLFE.

Regeneration

At the time of his death, LOEB had in the hands of his publishers a small book on regeneration[7] which will probably stand as his final, but certainly not his greatest contribution to biology. The book deals with the problems of regeneration in *Bryophyllum calycinum*, a plant which had been a favorite subject of research with the author for a number of years. The discussion is divided into two parts, the first of which considers the problem of mutilation and regeneration, and the second deals with the factors concerned in polarity of regeneration.

The general conclusions reached from the eight short chapters in part I is that a mass relation can be used to explain the amount of regeneration that occurs. Stated briefly, this mass relation is that equal masses of isolated tissues of the same type (as sister leaves or pieces of stem) under equal conditions of light, temperature, etc., produce about equal masses of regenerated roots and shoots in an equal length of time. The inhibiting action of the growth of one group of bud anlagen upon another group is found in the fact that sap and nutrients start to flow to the group that starts growth, and the continuation of this flow to the growing organs prevents the other anlagen from obtaining the nutrients and sap necessary for growth initiation. Gravity also affects the course of regeneration by causing the nutrients to percolate to the lower edge of a leaf suspended vertically on edge, so that the lower anlagen received the nutrients brought down by gravitation.

[7] LOEB, JACQUES, Regeneration. Pp. xii+143. New York: McGraw-Hill. 1924.

With reference to polarity, two possibilities are recognized, first that there might be a chemical difference between the ascending and descending sap which determines the nature of growth, and second, that the anlagen reached by ascending and descending sap are primarily different. LOEB decides in favor of the second alternative, since it was possible to secure regeneration of roots with both ascending and descending saps, and certain mass relations tended to corroborate this view.

The student of regeneration will be struck by the rather naïve and superficial experimentation and philosophy which the author has developed in this book. It seems to the reviewer, indeed, that the book is much more a contribution to the subject of nutrition and growth than to an understanding of regeneration. The main conclusion, that it requires a definite amount of nutrients to grow a definite amount of dry matter, is too obvious to need a book to set it forth. The observations recorded and the philosophy developed leave us about as much in the dark as ever upon the fundamental problems of regeneration.—C. A. SHULL.

The ferns

BOWER[8] has begun the presentation of the Filicales in the series of Cambridge Botanical Handbooks. This first volume deals with an "analytical examination of the criteria of comparisons," and is a detailed presentation of the facts and conclusions which he has been developing in previous publications. The subjects discussed will give a good idea of the contents. They are as follows: life history, habits and habitat, theoretical basis for systematic treatment, morphological analysis of shoot system, leaf architecture, cellular construction, vascular system of axis, vascular system of leaf, size a factor in stelar morphology, dermal and other non-vascular tissues, spore-producing organs (sorus and sporangium), gametophyte and sexual organs, embryo, abnormalities of life cycle, organographic comparison with other plants.

As may be inferred from this list of subjects, the volume is a treasure house of facts concerning the structure of Filicales, accompanied by suggestive conclusions. It certainly should be consulted by all morphologists. The theory of the "primitive spindle" is applied in the interpretation of structures, showing that filamentous structure is inherent in embryology, although frequently disguised. The conclusion is that such considerations "throw some welcome light upon the general architecture of the plant body, and point in the direction of a filamentous origin for even the most complex sporophytes."—J. M. C.

New Zealand plants

COCKAYNE[9] has given, in a very concise and instructive form, the results of his personal experience of 35 years in the cultivation of the native plants of New

[8] BOWER, F. O., The ferns (Filicales). Vol. I. Cambridge Botanical Handbooks. 4to. pp. 359. *figs. 309.* 1923.

[9] COCKAYNE, L., The cultivation of New Zealand plants. Auckland: Whitcombe and Tombs.

Zealand. The number of plants included exceeds 800. The habitat, or "place of dwelling" as COCKAYNE calls it, has been given special attention. There are 24 excellent photographs, a map, and a very attractive frontispiece representing the "crimson Manuka" (*Leptospermum scoparium* var. *Nichollsii*). The book deals with the methods of procuring and cultivating the plants, and has chapters on trees, shrubs, and climbing plants suitable for gardens, also on ferns for the open garden, plants for house decoration, native plants for school grounds and children's gardens, and town gardening. One paragraph may be quoted:

"Though the wild New Zealand plants live in all kinds of situations, and thrive under conditions unknown in gardens, it must not be thought that the majority are not amenable to cultivation. Quite the contrary is the case; only a small minority refuse to change their abode. Yet there is a deep-seated conviction to the contrary, notwithstanding the fact that many species have been cultivated for years."—R. B. THOMSON.

MINOR NOTICES

Shrubs of Indiana.—DEAM[10] has published a noteworthy contribution to the flora of Indiana, under the auspices of the Department of Conservation. The *Trees of Indiana* had been published previously, and developed a demand for a similar publication dealing with the shrubs. DEAM, as State Forester, has made a detailed study of the whole state, and the published results, therefore, are very complete. The classification, descriptions, and distribution are well presented, and in such a way that the non-professional user of the book may recognize the plants, especially helped by the numerous excellent plates.

It is the author's claim that the real value of shrubs is not appreciated. On this basis he divides them into three classes: those of ornamental value, those used in medicine and the industries, and those whose fruit is used. It is a credit to the Department of Conservation that it provides for the publication of such contributions.—J. M. C.

NOTES FOR STUDENTS

Gregarious flowering.—The simultaneous flowering at long intervals of many individuals of the same species has been recorded in many tropical and subtropical lands. The most remarkable record, extending (with some interruptions) back to 292 A.D., is that of a bamboo, *Phyllostachys puberula*, a native of China and Japan. All the individuals of this plant, however widely separated, have bloomed simultaneously every 60 years and then have died, and been replaced by seedlings. *Bambusa arundinacea*, of India, is known to flower simultaneously every 32 years, while some of the bamboos of southern Brazil are said to blossom at intervals of 13 years. SEIFRIZ[11] has recently shown that a bamboo of the West Indies, *Chusquea abietifolia*, has a cycle of 33 years.

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[10] DEAM, C. C., Shrubs of Indiana. Ind. Dept. Conservation Publ. 44. pp. 351. *pls. 146*. 1924.

[11] SEIFRIZ, W., The length of the life cycle of a climbing bamboo. A striking case of sexual periodicity in *Chusquea abietifolia* Griseb. Amer. Jour. Bot. 7:83–94. 1920.

Although such periodicity has been attributed to drought stimulus, SEIFRIZ[12] presents evidence that this hypothesis is without supporting data, and shows that it has been impossible to connect it with any climatic phenomena. Further, PETCH[13] has shown that such simultaneous flowering does not characterize all bamboos, although it may include species other than those cited. In particular he presents data to show that the giant bamboo, *Dendrocalamus giganteus*, does not flower gregariously.

A somewhat similar case is described by both SEIFRIZ[14] and PETCH (*loc. cit.*) in the simultaneous flowering of the palm *Corypyha umbraculifera*. On analysis by the latter author, it would seem that these palms reach a condition of maturity in 37 to 43 years, and that in Ceylon it is common to have groups of several or many individuals flower simultaneously every few years.

Another still more remarkable instance is recorded by PETCH of the gregarious flowering in Ceylon of several species of *Strobilanthus*, small trees of the Acanthaceae. They cover large tracts in the hill country, where several species flower and die simultaneously at intervals of 12 years. The different areas occupied by these plants may be arranged in two series, each maintaining its 12 year cycle, but with an interval of 5 (or 7) years between the times of general flowering and death of the two series. This has caused the erroneous assumption that *S. sexennis* flowers every 6 years. The situation is made still more complex by the behavior at Peradeniya of *S. viscosus*, which has flowered at intervals of 8 years, and it has been quite impossible to connect any of the flowering of *Strobilanthus* with any external factors.

The pigeon orchid, *Dendrobium crumenatum*, seems to present a still more complicated situation, for it has been observed to flower gregariously at very irregular intervals, but without the death of the plants after flowering. A record extending over a period of 4 years gave 27 flowering periods occurring at intervals varying from 10 days to several months. Recent investigations by SEIFRIZ[15] at Buitenzorg, Java, confirm the conclusions formerly reached by BURKILL, from data obtained in the Straits Settlements, that the flowering always came 8 days after a heavy rainfall. This gregarious flowering of orchids differs from the other instances cited, since it involves individuals of quite different ages, and seems to be the only case in which such flowering has been related to external stimulus.—GEO. D. FULLER.

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[12] SEIFRIZ, W., Observations on the causes of gregarious flowering in plants. Amer. Jour. Bot. **10**:93–112. *pl. 1.* 1923.

[13] PETCH, T., Gregarious flowering. Ann. Roy. Bot. Gard. Peradeniya **9**:99–117. 1924.

[14] SEIFRIZ, W., The gregarious flowering of the talipot palm, *Corypha umbraculifera*, at Peradeniya, Ceylon. Bull. Torr. Bot. Club **51**:341–350. *pls. 11.* 1924.

[15] ———, The gregarious flowering of the orchid *Dendrobium crumenatum*. Amer. Jour. Bot. **10**:32–37. 1923.

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Taxonomic notes.—RYDBERG[16] has published the result of his studies of *Odontotrichum*, recognizing 35 species, 6 of which are described as new.

BLAKE[17] has described 13 new species of *Verbesina*, bringing the number of species in this genus to 185, making it far the largest genus in its subtribe, and, with the exception of *Bidens*, the largest in the tribe Heliantheae.

GRESS[18] has published a very useful manual of the grasses of Pennsylvania. In the prefatory pages there are suggestions to amateur botanists, a statement of the genetic relationships of grasses, an explanation of their classification, and a full description of their gross anatomy. About 250 species and varieties are presented, in 70 genera, with abundant text illustrations to help in identification.

Recent contributions from the Gray Herbarium contain the following papers: PENLAND[19] has presented a very detailed account of the North American species of *Scutellaria*, recognizing 17 species, including several new combinations. FERNALD[20] has published 5 short papers, dealing with *Polystichum mohrioides* and other subantarctic or Andean plants in the northern hemisphere; the dwarf Antennarias of Northeastern America (11 species, 2 of which are new); the eastern representatives of *Arnica alpina* (8 species, one of which is new); some species of *Senecio* of eastern Quebec and Newfoundland; and new or recently restudied plants of eastern America (including a new species of *Agoseris* from the Quebec region). ROBINSON[21] has published some preliminary records to a general presentation of Eupatorieae. They include 2 new species of *Ophryosporus*, 16 of *Eupatorium*, and 11 of *Mikania*. In the same contribution JOHNSTON has published some new plants of Portuguese West Africa, 12 species in as many genera. The same author considers certain South American Proteaceae (one new species), and also presents a continuation of his studies of the Boraginaceae, defining and describing the "Old World genera" (60 in number), among which 4 new genera are established (*Microcaryum*, *Amblynotus*, *Oreogenia*, *Chionocharis*). He also publishes notes on miscellaneous American Boraginaceae.

FASSETT[22] has investigated the two species of *Zizania* (*Z. palustris* and *Z. aquatica*) recorded in GRAY'S *Manual*, with the following result. The species are *Z. aquatica* with three varieties (2 of them new) and *Z. latifolia*.

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[16] RYDBERG, P. A., Some Senecioid genera. II. Bull. Torr. Bot. Club **51**:409–420. 1924.

[17] BLAKE, S. F., New South American Verbesinas. Bull. Torr. Bot. Club **51**:421–436. 1924.

[18] GRESS, E. M., The grasses of Pennsylvania. Penn. Dept. Agric. **7**:245. 1924.

[19] PENLAND, C. W., Notes on North American Scutellarias. Contrib. Gray Herb. no. 71. 1924.

[20] FERNALD, M. L., *idem*, no. 72. 1924.

[21] ROBINSON, B. L., *idem*, no. 73. 1924.

[22] FASSETT, N. C., A study of the genus *Zizania*. Rhodora **26**:153–160. 1924.

Z. palustris disappears as a variety of *Z. aquatica*, and *Z. latifolia* emerges from a confused synonymy.

NELSON[23] has published 4 new species of *Phlox*, 2 new genera of Compositae (*Eremohylema* and *Calhounia*), and 8 miscellaneous new species. The new genus *Calhounia* contains 14 species previously assigned to *Nocca* or *Lagascea*.

PAYSON[24] has published a new genus (*Scoliaxon*) of Mexican Cruciferae; the species of *Pentstemon* native to Wyoming (33 in number); and also critical notes on various other groups.—J. M. C.

Photosynthesis.—A new method of estimating the amount of photosynthetic activity in marine algae has been employed by MOORE, WHITLEY, and WEBSTER[25] in a study of the influence of intensity and quality of light upon the rate of photosynthesis in green, brown, and red algae. As the bicarbonates are utilized in synthesis, the seawater becomes more alkaline. The degree of alkalinity change is easily determined by titration with phenolphthalein as indicator. The results they obtain by this method confirm the well-known facts that light from different regions of the spectrum from red to ultra violet has power to cause synthesis, but that the red rays are the most effective.

The distribution of the green, brown, and red algae in coastal waters is such that each kind is growing in the light intensity most favorable to the activity of its own chromophyll. Thus the green algae, with chlorophyll, are able to synthesize more rapidly than red algae when both are in strong light; but the red are more active than the green when both are in weak light. Moreover, the red forms synthesize carbohydrates more rapidly in weak light than when exposed to full sunlight. The brown forms are between the green and red in activity. Correspondingly each type is found growing at the depth which gives the light intensity most favorable to its own synthesis, the green algae near the surface, the brown somewhat deeper, and the red most deeply submerged. The red and brown pigments are held not to act as mere passive color screens, but as catalysts of photosynthesis in the same way as chlorophyll.

Studies of seashore pools show that the same changes in alkalinity occur in nature as in their experiments in vitro. The seasonal changes in hydroxyl ion concentration of the littoral waters may play an important part in the annual and seasonal development and succession of life in such habitats.—C. A. SHULL.

Permeability.—Using artificial cells as well as living and dried plant tissue, MACDOUGAL[26] has studied the permeability relations of the membranes. The

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[23] NELSON, AVEN, Taxonomic studies. Univ. Wyoming Publ. Bot. 1:47–68. 1924.

[24] PAYSON, E. B., Miscellaneous papers. Univ. Wyoming Publ. Bot. 1:69–108. 1924.

[25] MOORE, B., WHITLEY, ED., and WEBSTER, T. A., Studies of photosynthesis in marine algae. Trans. and Proc. Liverpool Biol. Soc. **37**:38–51. 1923.

[26] MACDOUGAL, D. T., The arrangement and action of material in the plasmatic layers and cell walls of plants. Proc. Amer. Phil. Soc. **63**:76–93. *figs. 2.* 1924.

artificial cell was essentially the same as that used in previous investigations. It consisted of a cellulose extraction thimble infiltrated and coated with substances present in the living cell. Thus by the addition or subtraction of a substance, the effect of this substance on permeability could be studied. As the term permeability is used by MACDOUGAL, a decrease in permeability denotes a change in the colloidal condition of the cell wall and plasmatic membranes, so that only water and the more active ions pass through, while in cases of an increase in permeability the largest molecules pass through. The effects of the salts of the common bases on permeability were studied, and results were obtained substantiating RABER'S conclusion that the relative density of the charge on the kation and anion of the salts is the thing to consider. Data concerning the effects of acids, bases, and salts on the swelling of agar and gelatin are given. Then various combinations of these substances and also pectin and lecithin were used in the artificial cell, and similar results obtained. For example, in the experiments on the effect of acids on the swelling or hydration of agar, increase in acidity caused decrease in hydration. A decrease in hydration means decrease in permeability, and in the case of the special cell, as the acidity increased the amount of endosmose or turgidity increased. Studies were made on the effect of acids, bases, and salts on the swelling of living and dried slices of *Opuntia*, the plasmatic masses of which have a rather high pentosan content. Finally, data on the effect of the concentration of the cell sap on the absorption of ions were recorded. The results obtained support the common idea that the osmotic concentration of the cell sap has little to do with the intake of ions.

Studies such as these of MACDOUGAL are doing much to increase our knowledge of the real nature of permeability.—S. V. EATON.

Hydrogen ion concentration.—Using colorimetric methods, ATKINS[27] has studied the hydrogen ion concentration of seawater. He finds that seawater may become as alkaline as P_H 9.7, due to very active photosynthesis. The maximum alkalinity was found in May and August, with a minimum in July. The maximum in May was found to correspond with the maximum average number of hours of sunshine daily, rather than with the maximum length of day, which would come of course in June. Data obtained from changes in the P_H value of the seawater are used to calculate the minimum amount of carbohydrates synthesized in the sea. The actual amount of carbohydrates found is the result of course of an equilibrium between production by photosynthesis and destruction by plant and animal respiration. It is stated that in a general way the P_H maxima may be correlated with the diatom maxima in early summer and autumn. Perhaps further work will show the P_H concentration a rather useful criterion in studying the distribution of algal life.—S. V. EATON.

[27] ATKINS, W. R. G., Hydrogen ion concentration of seawater in its biological relations. Pt. I. Jour. Marine Biol. Assn. **12**:717–771. 1922.

———, Hydrogen ion concentration of seawater in its relation to photosynthetic changes. Pt. II. Jour. Marine Biol. Assn. **13**:93–118. *figs. 11.* 1923.

Determination of chlorides.—LAWRENCE and HARRIS[28] have developed a method of determining the chloride content of plant tissue fluids which seems accurate and rapid. Essentially the method consists of the use of a known excess of silver nitrate to precipitate out the chlorides, the destruction or altering of the proteins or other organic compounds which might fix a certain amount of the silver by boiling the fluids with concentrated nitric acid, and finally the titration of the excess silver nitrate against potassium thiocyanate solution, ferric alum being used as an indicator. The nitric acid has the effect also of decolorizing the fluids and of freeing any chlorine that may be in organic combination. Writers on technique quite generally warn against the titration of the excess silver in the presence of the silver chloride precipitate, but the authors show that in the presence of nitric acid the silver chloride forms rather large masses, so that on account of the small surface exposed the reaction between the silver chloride and the potassium thiocyanate is negligible. In this way there is eliminated the most time-consuming part of the method as it has usually been practised.—S. V. EATON.

Light requirement of trees.—Using as a standard the amount of electricity generated by the light falling on a thermo-couple and measured by a sensitive galvanometer, BURNS[29] has determined the minimum light requirement of several forest trees more exactly than has previously been done. He used two strong nitrogen filled electric bulbs as a source of light, and secured differences in exposure by varying the distance between the light and the trees. Young trees 18–24 inches in height were used inclosed in bell jars, and the minimum light requirement was regarded as that which gave a respiration-photosynthesis coefficient slightly above unity, as determined by gas analysis. The minimum light requirement determined for 14 species was: *Pinus ponderosa* 306, *P. sylvestris* 287, *Thuja occidentalis* 186, *Larix laricina* 176, *Pseudotsuga mucronata* 136, *Pinus Murrayana* 136, *Quercus rubra* 133, *Celtis occidentalis* 115, *Picea Engelmannii* 106, *Pinus Strobus* 104, *Picea excelsa* 87, *Tsuga canadensis* 84, *Fagus grandifolia* 75, and *Acer saccharum* 34.—GEO. D. FULLER.

North American flora.—The fourth part of volume 24 contains a continuation of the Galegeae (Fabaceae) by RYDBERG, including 25 genera, with 117 species, 17 of which are described as new. The large genera are *Robinia* (20 species, 2 of which are new), *Benthamantha* (19 species, 8 of which are new), and *Diphysa* (17 species, 5 of which are new). The remaining 61 species are distributed among 21 genera.

[28] LAWRENCE, J. V., and HARRIS, J. A., Tests of a wet oxidation and modified Volhard method for the determination of chlorides in plant tissue fluids. Jour. Amer. Chem. Soc. **46**:1471–1477. 1924.

[29] BURNS, G. P., Studies in tolerance of New England forest trees. IV. Minimum light requirement referred to a definite standard. Vermont Exp. Sta. Bull. 235. pp. 32. 1923.

The ninth part of volume 7 contains the conclusion of Aecidiaceae (Uredinales) by ARTHUR. Four genera are presented: *Uredo*, with 60 species (2 new); *Aecidium*, with 84 species (4 new); *Peridermium*, with 7 species (2 new); and *Caeoma*, with one new species. It is interesting to note that the author's name is associated with 32 species of *Uredo* and 43 species of *Aecidium*. There are 20 pages of additions and corrections to Uredinales, including a new genus (*Synomyces*) based on *Coleosporium Reichei* Dietel.—J. M. C.

Embryogeny.—SOUÈGES,[30] in continuation of his unremitting work on the embryogeny of angiosperms, has published an account of *Sagina procumbens* (Caryophyllaceae), with interesting conclusions. He has also published, in the Proceedings of Académie des Sciences for 1924, abstracts of his results with the following forms: *Polygonum aviculare* (January), *Poa annua* (March), *Linum catharticum* (April), *Sherardia arvensis* (June), *Sparganium simplex* (July), and *Euphorbia Esula* (November).—J. M. C.

North American Flora.—The fourth part of volume 10 continues the presentation of the Agaricaceae, including *Inocybe* by C. H. KAUFFMAN and *Pholiota* by L. O. OVERHOLTS. *Inocybe* includes 105 species, 16 of which are new; and *Pholiota* includes 48 species, 2 of which are new. It is interesting to note that 28 species of *Inocybe* are credited to PECK, and 20 species to ATKINSON. In *Pholiota* 19 species are credited to PECK.—J. M. C.

Morphological studies of Scrophulariaceae.—HOLM[31] has published a very detailed account of the anatomical structure of the roots, stems, and leaves of *Ilysanthes*, *Scrophularia nodosa* var. *americana*, and *Linaria canadensis*, especially of the two latter species. The details are too numerous to mention, but such data are very valuable for comparative studies of responses and relationships.—J. M. C.

[30] SOUÈGES, R., Développment de l'embryon chez le *Sagina procumbens* L. Bull. Bot. France IV. **24**:590–614. *figs. 48*. 1924.

[31] HOLM, THEO., *Ilysanthes*, *Scrophularia*, and *Linaria;* a morphological study. Amer. Jour. Sci. V. **8**:395–410. 1924.